The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 14 JULY, 1954 NUMBER 7
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URINARY EXCRETION OF HISTAMINE AFTER ADMINISTRATION OF CORTISONE*

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ADMINISTRATION of cortisone reduces the concentration of histamine in the blood of guinea pigs, dogs and human beings (1). The present study was undertaken to determine whether oral administration of cortisone would affect the excretion of histamine in the urine of healthy human beings.

METHODS AND PROCEDURES

Free and conjugated forms of histamine were determined in the urine by the method of Roberts and Adam (2). The mean recovery of histamine by this procedure is about 67 per cent (2, 3) and, if desired, a factor may be applied to correct for the loss. The results given in the present paper, however, are uncorrected and are expressed in terms of the free base of histamine. In all instances, duplicate samples of urine were used for determination of the concentration of both free and conjugated histamine.

In each test the urine passed during twenty-four hours was collected in a chemically clean bottle of 2-liter capacity containing 10 ml. of twice normal hydrochloric acid to maintain the urine at a pH of 5 or less. The urine was stored in a refrigerator until the extraction of histamine was started; in all cases this was within twenty-four hours of

Received for publication November 6, 1953.

* Abridgment of thesis submitted by Dr. Mitchell to the Faculty of the University of Edinburgh, Edinburgh, Scotland, in partial fulfillment of the requirement for the degree of Doctor of Medicine. Work represented in this paper was carried out at the Mayo Foundation, Rochester, Minnesota.

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completion of the collection. Previous studies have shown that no loss of histamine occurs under these conditions.

Five healthy men were studied. The 24-hour urinary excretion of free and conjugated histamine of each person was assayed for eleven consecutive days. Throughout this period the volunteers were asked to eat a mixed diet containing more or less constant amounts of meat each day. The collections obtained during the first three days of the tests were used as a control for the fourth, fifth and sixth days, during which each person took 50 mg. of cortisone by mouth every six hours (200 mg. per day). Daily observations were then continued for five more days. This sequence was followed twice with the first volunteer and once with the other 4.

RESULTS

The 24-hour output of free histamine in the urine increased above the control values during or immediately after the period of administration of cortisone in each of 6 tests on 5 subjects (Table 1). In 5 of the tests, the peak value occurred on a day during which cortisone was being taken. The mean 24-hour excretion of free histamine was calculated for the con-

TABLE 1. EFFECT OF CORTISONE ON URINARY EXCRETION OF FREE AND CONJUGATED HISTAMINE

Consecutive days	Urinary histamine, μg. per 24 hours											
	Subject I				Subject II		Subject III		Subject IV		Subject V	
	Test a		Test b									
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
1	24.0	24	25.5	149	21.0	18	20.8	23	9.5	25	19.0	18
2	16.1	13	26.8	103	18.9	20	18.0	46	10.7	52	19.9	32
3	20.6	15	23.2	38	21.6	54	16.1	17	12.4	153	19.6	29
4*	25.8	13	20.9	35	25.9	22	23.2	46	15.7	39	12.8	34
5*	26.8	15	24.2	26	28.0	17	25.3	39	13.4	14	31.4	22
6*	30.9	24	25.4	25	33.9	40	25.6	26	16.4	11	17.7	25
7	19.2	37	29.2	47	18.3	16	17.6	16	7.7	13	24.9	33
8	20.7	30	18.5	120	28.1	64	21.4	20	8.7	33	24.6	35
9	21.8	17	22.8	29	25.5	85	20.3	21	12.3	60	25.9	27
10	15.6	15	15.8	30	21.4	95	14.2	21	9.4	35	17.5	19
11	16.7	16	17.8	32	20.4	67	15.9	24	9.8	30	20.7	33

* Cortisone (200 mg. per day) given.

trol period and for the period of administration of cortisone in each test. Comparison of the differences between these means indicated that a significant increase had occurred in the excretion of free histamine during the period when cortisone was given (p less than .05).

The general pattern of the changes is delineated most clearly when the results are plotted as a percentage of the control values (Fig. 1). The increasing excretion of histamine that occurred in most of the tests during the three days when cortisone was given is clearly apparent. The results in Subject IV appear to be an exception but the control values obtained

in this subject were high (Table 1). Indeed, the values in this particular test were greater than any obtained in other tests on this subject. The high excretion under control conditions accounts for the low percentages appearing in the figure. Although the range of the values is low, the pattern of the changes is nevertheless the same as in the other tests shown. Within

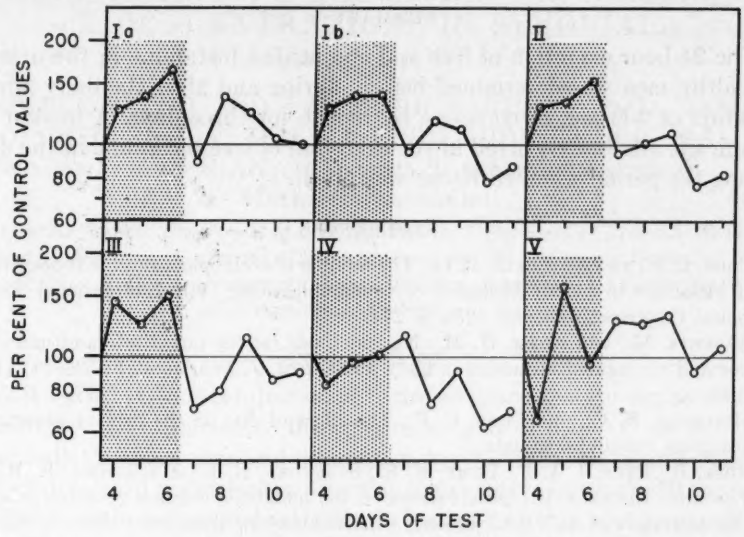

FIG. 1. Effect of administration of 200 mg. of cortisone by mouth for three days on the excretion of free histamine in urine of 5 healthy men. In each subject, the mean 24-hour excretion during the three consecutive days just before the administration of cortisone was used as a control value, and the amounts of free histamine excreted in subsequent days are expressed as a percentage thereof. The values are plotted on the chart in a logarithmic scale. The shaded area delineates the three days during which cortisone was given.

five days after cessation of the administration of cortisone, the excretion of free histamine in the urine of all of the volunteers had returned to within the range of the control values. Fluctuations in the amounts of conjugated histamine excreted in the urine during the control period were so great that no changes occurring in subsequent periods could be definitely attributed to the administration of the hormone (Table 1).

COMMENT

A transitory increase in the total output of histamine in the urine has been noticed by Rose and associates (4) and by Grob (5) when corticotropin (ACTH) or cortisone was given to patients who had various allergic diseases. The measurement of total urinary histamine in these instances

included both the free and the conjugated forms. The present study demonstrates that a modest increase in the urinary excretion of free histamine occurs in healthy persons when cortisone is taken by mouth. The additional free histamine in the urine may arise as a result of its liberation in the body, its increased metabolic turnover or its reduced destruction.

SUMMARY

The 24-hour excretion of free and conjugated histamine in the urine of 5 healthy men was determined before, during and after the daily administration of 200 mg. of cortisone by mouth for three days. A modest but significant increase occurred in the excretion of free histamine in the urine during the period when cortisone was given.

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DISPOSITION OF INTRA-ARTICULARLY INJECTED HYDROCORTISONE ACETATE, HYDROCORTISONE AND CORTISONE ACETATE IN ARTHRITIS. I. CONCENTRATIONS IN SYNOVIAL FLUID AND CELLS*

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INTRODUCTION

INTRA-ARTICULAR injection of hydrocortisone acetate has been demonstrated by Hollander, Brown, Jessar and Brown (1) to produce prompt alleviation of pain and diminution of synovial swelling and effusion with a fall toward normal intra-articular temperature in nearly 90 per cent of patients with rheumatoid arthritis. In contrast to this, cortisone acetate in similar or even larger doses is far less effective, with only about 25 per cent of the patients showing even partial improvement after intra-articular injection.

The reason for this difference in local anti-inflammatory action is obscure. Original assumptions that the difference was due to lower solubility of hydrocortisone acetate have proved incorrect, since more soluble forms of hydrocortisone have proved as effective as the acetate, and less soluble esters of cortisone no more effective than the cortisone acetate (1).

These studies were undertaken to determine whether there was a significant difference in the rates of change in concentration of the hormones in the synovial fluid, and possible differences in concentrations in the synovial fluid cells and the structures of the joint itself. The purpose of this report is to present data on concentrations of hormone in the synovial fluid and the distribution between cells and fluid at various intervals after intra-articular injection, as well as the rate of hydrolysis of the acetate esters of cortisone and hydrocortisone.

Received for publication November 18, 1953.

* Supported by a grant from the Helon Augusta Parkhill Foundation.

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METHODS

Clinical

Sixteen patients with active arthritis of both knee joints were selected for the initial study. Four patients had osteoarthritis and 12 had rheumatoid arthritis. In 16 instances, injections were made into both knee joints and in 6 instances into only one. In 2 patients, four paired experiments were performed. In all, hydrocortisone acetate was injected 16 times, cortisone acetate 15 times, and free hydrocortisone 7 times.

Cortisone acetate ("E"ac) and hydrocortisone acetate ("F"ac) were injected intra-articularly in doses of 25 mg., whereas hydrocortisone ("F") was given in approximately isomolecular amounts (22.5 mg.). The hormone suspension was thoroughly mixed with the synovial fluid of the knee, both by a repeated withdrawal and re-injection ("pumping") at the time of injection, and by immediate active motion of the knee joint. Samples of synovial fluid (1 to 5 ml.) were withdrawn within approximately three minutes for determination of "initial" concentration of hormone. The subjects were permitted to move about moderately during the observation period but excessive exertion was not permitted. At varying intervals following the injections, samples of synovial fluid were again aspirated for analysis, and clinical observations were made of possible changes in the degree of inflammation of the joints under study.

Laboratory

Extractions were made with chloroform on an automatic shaker at pH 1 for one hour. The concentration of "17-hydroxycorticosteroids" (17-hydroxycorticoids) in the joint fluid and cells was measured by a modification of the method of Porter and Silber (2) and of Nelson and Samuels for blood (3). The modification resulted in the production of the same color density by both hydrocortisone and cortisone. The cells were separated from the fluid by centrifuging 3 ml. of joint fluid and washing the centrifugate with physiologic saline. To destroy the cells, they were then suspended in 30 ml. of water and exposed to ultra sound (9,000 cycles per second in a magneto striction oscillator for 15 minutes; Submarine Signal Company, type R-221). After extraction, the amounts of 17-hydroxycorticoids were then determined for the "cells," supernatant liquid and washings.

The accuracy of the method was examined by comparing the 17-hydroxycorticoids determined on the total uncentrifuged fluid with the sum of the values found for "cells," supernatant fluid and washings in 17 paired observations obtained within seventy-five minutes of injection. The average deviation of the observations from the mean of each pair was 4.3 per cent, with a maximum deviation of 14 per cent. This degree of accuracy is believed to be due to the relatively high concentrations in the joint fluid during the first few hours after injection. It also suggests that destruction of the "cells" was not necessary for extraction of the steroids.

The relative proportion of the free form and the acetate present in the fluid and in the cells was determined by chromatographing appropriate aliquots of extract by a

modification of the method of Burton, Zaffaroni and Keutmann (4). The extraction method used was demonstrated not to hydrolyze the acetates of hydrocortisone and cortisone. The finding of reduced blue tetrazolium at the appropriate positions on the paper chromatograms for cortisone and hydrocortisone and their acetates was considered presumptive evidence that these compounds were responsible for the reduction of the blue tetrazolium. Approximations of quantities on the chromatogram were made by the method of Tennent, Whitla and Florey (5).

RESULTS

Clinical

In all but one instance, there was a clinically appreciable decrease in the degree of inflammation of the arthritic knee injected with hydrocortisone acetate or free hydrocortisone, which became apparent between six and eighteen hours following the injection and which persisted for four days to one month. No such marked reduction in pain or swelling was noted in the knee joints into which cortisone acetate had been injected. Although in 5 instances, the patient noted some decrease in pain in the cortisone-treated knee also, this improvement lasted a maximum of forty-eight hours.

Experimental

a. *Concentration of 17-hydroxycorticoids at various time intervals after intra-articular injection.* Figure 1 shows the concentration of 17-hydroxycorticoids in uncentrifuged specimens of joint fluid at various time intervals up to 180 minutes after intra-articular injection of 25 mg. of cortisone acetate, of hydrocortisone acetate, and of free hydrocortisone. In general, the slopes of the lines indicating the percentage decrease in concentration against time are essentially the same for cortisone acetate, hydrocortisone acetate and hydrocortisone up to 180 minutes after injection in any individual subject. The six paired experiments in separate individuals in which hydrocortisone acetate (25 mg.) was injected into one knee joint and the same amount of cortisone acetate into the other were analyzed statistically.[1] There was no evidence of a significant difference in the rate of decrease (in percentage of initial concentration) of 17-hydroxycorticoids in the joint fluid. The rate of decrease in concentration after injection of hydrocortisone was 5.7 per cent $\pm$ 1.7 per cent per minute; after hydrocortisone acetate, 3.6 per cent $\pm$ 1.5 per cent per minute; and after cortisone acetate, 4.0 per cent $\pm$ 2.2 per cent per minute. The standard errors of the differences between the means indicate that there is a greater chance than 1 in 20 that the differences were due to chance alone. However, the relative values of the means suggest that the rates of change in concentration may

[1] We are grateful to Hyman Menduke, Ph.D., Instructor, Department of Economic and Social Statistics, University of Pennsylvania, for his aid in the statistical analysis of the data.

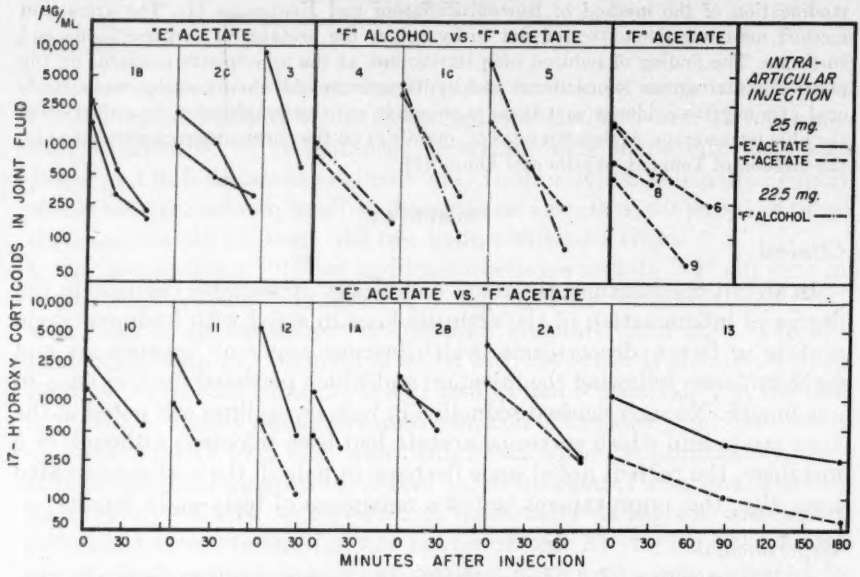

FIG. 1. *The change in concentration of 17-hydroxycorticoids in joint fluid after intra-articular injection.*

The results are plotted semi-logarithmically. Patients with bilateral arthritis of the knee joints (Nos. 1 to 5 and 10 to 13 inclusive) were injected intra-articularly with approximately isomolecular amounts of cortisone acetate ("E" acetate), hydrocortisone acetate ("F" acetate) or free hydrocortisone ("F" alcohol). Patients 6 to 9 inclusive were given unilateral injections. In Patients 1 and 2, the sequence of experiments is indicated by the letters. The concentrations of 17-hydroxycorticoids were measured at various intervals after injection. The initial specimens were obtained within approximately three minutes after injection. The percentile decrease in concentration, as indicated by the slope of the lines, is approximately the same for the three compounds and the rate of change in concentrations seems to be more closely associated with the individual subject than with the type of compound. (For statistical analysis, see text.)

Experiments 1d, 15, and 16, using the free form of hydrocortisone, are discussed in the text but not included in this chart.

be inversely related to the proportion of each material taken up by the cells (Fig. 2). More observations would be needed to test this possibility. In addition, the rates of change in concentration after injection of hydrocortisone acetate and cortisone acetate for the 3 paired experiments in Subject #1 (exp. a, b and c) were found to range from 4.8 per cent to 7.7 per cent per minute, whereas those for 3 paired experiments in Subject #2 after injection of the same steroids ranged from 1.9 per cent to 3.9 per cent per minute, and the rate for both injections in Subject #13 was 1.2 per cent per minute. It thus appears that variation in the rate of change in

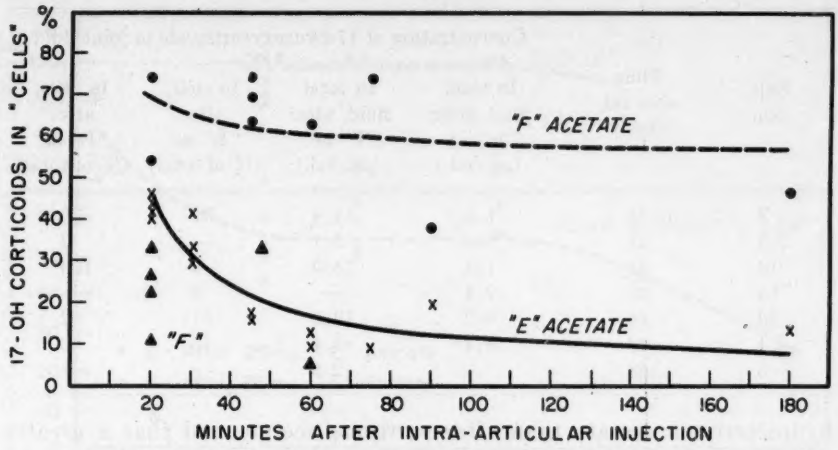

FIG. 2. *Distribution of 17-hydroxycorticoids in cells and fluid.*

The proportion of 17-hydroxycorticoids contained in the cells is expressed as a percentage of the concentration in the uncentrifuged joint fluid. After intra-articular injection of the free form of hydrocortisone ("F") (▲) only a small proportion is found in the cells; yet a considerably greater proportion of 17-hydroxycorticoids is found in the cells after injection of hydrocortisone acetate ("F" acetate) (●). After the injection of cortisone acetate ("E" acetate)(X), the proportion found in the cells changes rapidly with time. At twenty minutes after injection, it is evident that the proportion contained in the cells is less than that after hydrocortisone acetate injection and greater than that after hydrocortisone.

The regression equations were calculated by the method of least squares. They are as follows:

Percentage of 17-hydroxycorticoids in cells after cortisone acetate injection

$$=\left(3+\frac{810}{\text{time in minutes}}\right)\pm 6.$$

Percentage of 17-hydroxycorticoids after hydrocortisone acetate injection

$$=\left(56+\frac{262}{\text{time in minutes}}\right)\pm 13.$$

concentration is more closely associated with the physiologic differences in the subjects than with the chemical characteristics of the compounds.

Measurements were also made at 24, 48 and 168 hours after intra-articular injection in some experiments. Results were as shown in the tabulation on the next page.

The data show the low concentration of Porter-Silber reacting material found in uncentrifuged (total) joint fluid and cells 24 to 168 hours after intra-articular injection. It seems evident that the concentrations of Porter-Silber reacting material at these time intervals were greater after

Exp. No.	Time after inj. (hrs.)	Concentration of 17-hydroxycorticoids in joint fluid			
		In total fluid, after "E" ac. (μg./ml.)	In total fluid, after "F" ac. (μg./ml.)	In cells, after "E" ac. (% of total)	In cells, after "F" ac. (% of total)
3	24	1.5	13.4	37	70
5	24	—	5.1	—	2
13	24	1.1	25.0	0	100
13	48	2.4	—	0	—
2d	48	4.2	13.0	31	82
14	48	3.4	3.1	15	83
6	168	—	2.9	—	—

hydrocortisone acetate than after cortisone acetate and that a greater proportion of material was in the cells. It seems probable that this may be partially accounted for by the absorption of intra-articular fluid as a result of hydrocortisone acetate injection and the fact that hydrocortisone acetate appears to be taken up by the cells in greater proportion than cortisone acetate and hence may be less likely to pass through the synovial membrane.

b. *Distribution of 17-hydroxycorticoids in cells and fluid.* Figure 2 demonstrates that the proportion of 17-hydroxycorticoids present in the cells varies with time and the type of steroid injected intra-articularly. Most of the free form of hydrocortisone is present in the fluid, whereas the acetate is concentrated in the cells. Cortisone acetate shows a rapid decrease in the proportion found in the cells, being considerably less concentrated in the cells than hydrocortisone acetate, but more so than the free form of hydrocortisone acetate.

c. *The proportion of 17-hydroxycorticoids present as the acetate and free form in cells and fluid.* Figure 3 indicates that most of the material in cells for at least the first seventy-five minutes after intra-articular injection of cortisone acetate and hydrocortisone acetate is acetylated, but the supernatant fluid (plus washings) shows a rapid decrease in the proportion of 17-hydroxycorticoids present in this form.

DISCUSSION

No clear-cut explanation was found from these studies for the marked anti-arthritic effect of intra-articular injection of hydrocortisone and hydrocortisone acetate compared to the slight or nonexistent effect of cortisone acetate given by the same route. In agreement with Wilson *et al.* (6), we have found that the rate of change in concentration of 17-hydroxy-

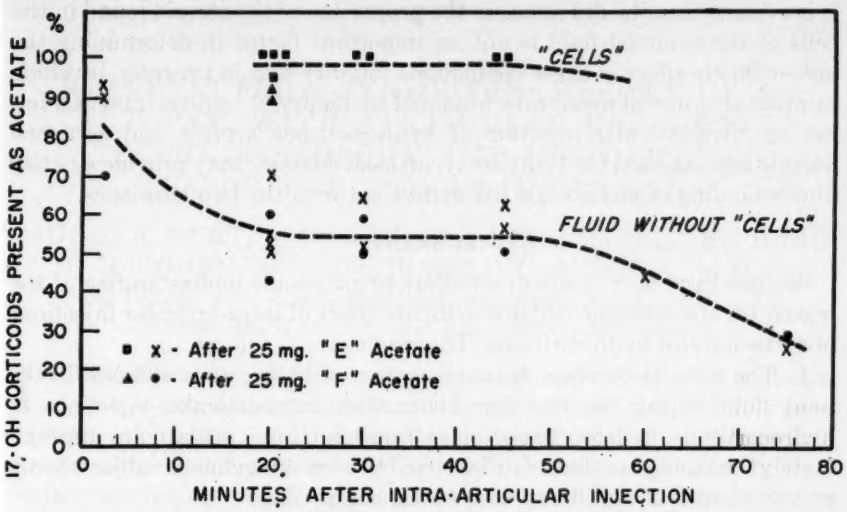

FIG. 3. *Proportion of 17-hydroxycorticoids present as the acetate in "cells" and fluid.* The material extracted from the cells and the supernatant fluid (plus washing of the cells) were chromatographed on paper and the relative proportions of the free compound and the acetate measured. The proportions of unhydrolyzed hydrocortisone acetate ("F" acetate) and cortisone acetate ("E" acetate) in the fluid decreased relatively rapidly during the first seventy-five minutes, whereas the proportion of the material present in the cells as the acetate remained high. The dotted lines are drawn free-hand.

corticoids in uncentrifuged joint fluid is approximately the same after injections of any of the three compounds. It seems probable, however, that further observations might indicate a moderate difference, even though it seems clear that the rate of fall, in the first few hours at least, is more closely associated with individual differences in the patient than with the chemical configuration. In addition, the proportion of hydrolyzed compared to unhydrolyzed materials in the cells or the cell-free fluid was essentially similar for both the acetates. Relatively little of the compound within the cells is present as the free form for at least the first seventy-five minutes, whereas approximately 40 per cent or more of the compound in the fluid is in the free form from the twentieth minute after injection of either cortisone acetate or hydrocortisone acetate.

The concentration of 17-hydroxycorticoids in the cells after injection of hydrocortisone acetate is considerably and significantly greater than that after injection of cortisone acetate. On the other hand, after the injection of free hydrocortisone, the concentration of 17-hydroxycorticoids in the cells appears to be even less than after cortisone acetate injection. Since both the free and acetylated forms of hydrocortisone are effective clinically,

it is evident that the difference in the proportion of the steroid found in the cells of the synovial fluid is not an important factor in determining the anti-arthritic effect of these compounds. Studies now in progress, in which samples of synovial membrane obtained by biopsy at various intervals following intra-articular injection of hydrocortisone acetate and cortisone acetate are analyzed for 17-hydroxycorticoid content, may provide a better understanding of physiologic differences between the two hormones.

SUMMARY

Studies have been made in an effort to gain some understanding of the reason for the difference in anti-arthritic effect of intra-articular injections of cortisone and hydrocortisone. The results are as follows:

1. The rates of decrease in concentration of 17-hydroxycorticoids in the joint fluid during the first few hours after intra-articular injections of hydrocortisone, hydrocortisone acetate and cortisone acetate are approximately the same, the effect of differences between some clinical subjects being greater than that of differences between compounds.

2. The hydrolyzed forms of both hydrocortisone acetate and cortisone acetate were present in greater proportions in the fluid than in the cells.

3. The proportion of 17-hydroxycorticoids present in the cells after injection of cortisone acetate is greater than after the injection of the free form of hydrocortisone, but less than after the injection of hydrocortisone acetate.

These data do not afford an explanation for the difference in anti-arthritic effect between hydrocortisone and cortisone. Further studies are in progress.

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KLINEFELTER'S SYNDROME: REPORT OF AN AUTOPSY, WITH PARTICULAR REFERENCE TO THE HISTOLOGY AND HISTOCHEMISTRY OF THE ENDOCRINE GLANDS*

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IN 1942 Klinefelter, Reifenstein and Albright (1) described a syndrome of male hypogonadism characterized by gynecomastia, aspermatogenesis without a-leydigism, and increased excretion of gonadotropic hormone. Emphasizing that breast development, hair growth, body build, and secondary sex organs might vary considerably with this testicular picture, Heller and Nelson (2) later expanded the concept of this syndrome to include all patients with small atrophic testes containing hyalinized tubules and clumped Leydig cells, azoospermia, and elevated urinary gonadotropins. This broader clinical concept has subsequently been accepted by Howard, Sniffen, Simmons and Albright (3). The present paper reports the autopsy of such a patient who had received no hormone therapy. The endocrine glands were examined by histologic and histochemical methods and the results related to descriptions in the literature of men whose testes fit Klinefelter's original description.

REPORT OF CASE

W. W. (BIH-M23477) was a 63-year-old man of English stock. Clinical study nine months before death revealed hypertension, left ventricular hypertrophy, and albuminuria. As the patient was also noted to have large breasts and a nodular goiter, an investigation of his endocrine status was undertaken.

Family history was noncontributory. The body weight was 78 Kg., height 170 cm., and habitus moderately eunuchoid. There was girdle fat distribution, the patient stating that his abdomen and breasts had always been prominent. No note was made as to his beard. Axillary hair was scanty and pubic hair of feminine distribution. The patient claimed to have normal libido and potentia, but his marriage of forty years' duration had remained childless. The penis was of normal size; both testes were fully descended and were small and firm. The thyroid was large and contained multiple olive sized nodules in the lower poles of both lobes.

Laboratory investigations: The basal metabolic rate was +12 and +2 per cent of nor-

Received for publication May 16, 1953.

* This investigation was supported in part by research grant No. C-1451 C2 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

mal on two occasions. The uptake of a tracer dose of radioactive iodine was in the euthyroid range (41 per cent). Urinary gonadotropin excretion was 47 mouse units per twenty-four hours.[1] Radiographic examination of the skull revealed all paranasal sinuses to be unusually large. In addition to mild but distinct hyperostosis frontalis interna, there were manifestations suggesting acromegaly—moderate prognathism, an enlarged occipital spur, and a large frontal process.

Course: The patient refused further investigation or any type of treatment and subsequently had congestive heart failure. He was admitted to the hospital because of an attack of right sided hemiplegia. Hemoglobin, nonprotein nitrogen, total proteins, albumin/globulin ratio, carbon dioxide combining power, serum chloride and serum potassium levels were normal. The white blood cell count was 14,350. The urine (specific gravity 1.020) contained 2 plus albumin. The serum sodium concentration was 146 mEq. per liter. The Hinton test gave negative results.

The patient lapsed into coma and died on the fifth hospital day.

Autopsy

Autopsy (BIH A52-32): The *heart* (480 Gm.) showed eccentric hypertrophy of the left ventricle and moderate coronary atherosclerosis. No mural thrombi were found in the heart. The *lungs* (1000 Gm.) showed emphysema and foci of pneumonia. The *kidneys* (each weighing 200 Gm.) were not remarkable except for a small fresh cortical infarct on the right. The *brain* contained three discrete areas of recent softening. The *arteries* at the base of the brain, although atherosclerotic, showed no significant narrowing.

Endocrine glands

Testis: Both testes were fully descended and of normal shape but measured only 1.6 cm. in longest dimension. The tunica albuginea was normal. The cut surface did not string and was a deep reddish brown peppered with minute white dots. The epididymides were decidedly larger than the diminutive testes.

Sections stained with hematoxylin and eosin showed masses of interstitial cells in which very few sclerotic tubules could be identified (Fig. 1). These tubules had been converted into cords made up of fine undulating collagen fibers. A basement membrane was not discernible and there was no peritubular fibrosis. No germ cells were found, but a few tubules contained atrophic Sertoli cells (Fig. 2).

The Leydig cells were aggregated in large, well vascularized masses. The individual interstitial cells were polymorphic. The predominant cell type was large and polyhedral with eosinophilic cytoplasm, fine refractile eosinophilic granules and large amounts of deep tan, finely and coarsely granular pigment (Fig. 3). Some cells contained large, spherical hyaline droplets. The eccentric nuclei contained granular chromatin; nucleoli were not visible. Some nuclei were excessively large and occasionally bizarre. The masses of Leydig cells were well vascularized.

In the surrounding loose connective tissue were cells having the configuration of fibrocytes and often containing abundant pigment. Collagen fibers were moderately increased in numbers. The arterioles, small arteries, veins, and the capsule appeared normal.

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[1] Method of Gregory Pincus (4). This does not differentiate between FSH and LH. The upper limit for normal men is 15 mouse units. The value of 47 mouse units is in the range for castrates and postmenopausal women.

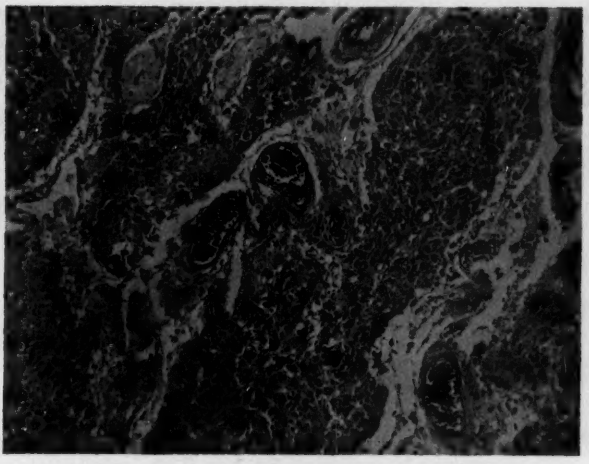

FIG. 1. Low-power view of testis. Masses of interstitial cells are separated by connective tissue septa. There are no recognizable tubules. Hematoxylin and eosin, ×78.

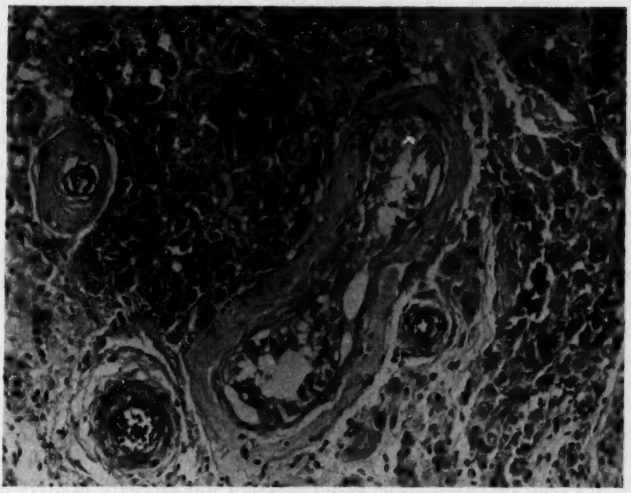

FIG. 2. Two hyalinized testicular tubules lined with vacuolated Sertoli cells and surrounded by masses of Leydig cells. Hematoxylin and eosin, ×90.

The Leydig cells differed from normal in the following respects: absence of cytoplasmic vacuoles and coarse granules, paucity of crystalloids, increased amounts of cytoplasmic pigment, occasional nuclear gigantism and distortion, absence of visible nucleoli, and relative increase in fibrocyte-like forms.

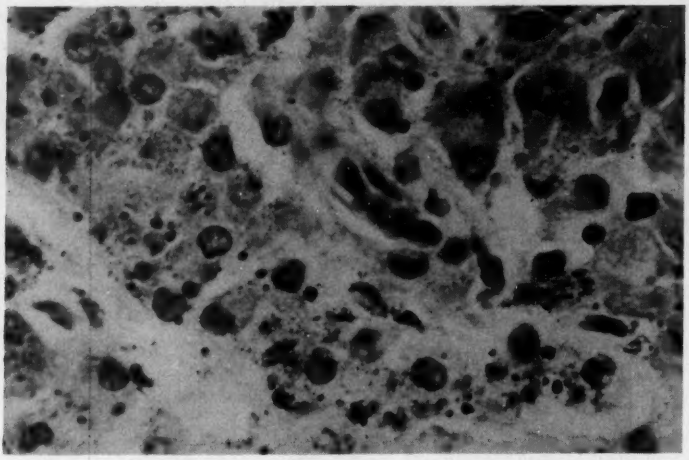

FIG. 3. Pigmented granules in Leydig cells. The black granules in the photograph were actually deep tan. Hematoxylin and eosin, ×513.

Histochemical studies of the interstitial cells revealed a small quantity of finely granular cytoplasmic sudanophilic material. In occasional cells large sudanophilic globules filled the cytoplasm. The Schultz and Schiff reactions were negative. No birefringence was noted before or after digitonin treatment. A very few of these cells contained sparse blue granules after the application of the Ashbel-Seligman technique (5). In the Sertoli cells cytoplasmic vacuoles contained small quantities of sudanophilic as well as Ashbel-Seligman positive substance.

Prostate: The prostate appeared to be enlarged, but no measurements were taken. It was symmetrical and contained no nodules. Microscopically there was no evidence of either hypertrophy or atrophy.

Adrenal: The adrenal glands appeared normal on gross examination but were not weighed. The zona glomerulosa and zona fasciculata appeared normal in hematoxylin and eosin preparations. In the zona reticularis many cells contained much brown pigment in clumps and fine granules. The medulla was not remarkable.

Upon histochemical examination, most of the cells of the zona fasciculata contained large and small sudanophilic droplets. Only small amounts of sudanophilic substance were found in the zona glomerulosa and reticularis. Similar in distribution, Schultz-positive lipoid, birefringent material, and birefringent digitonids were present in considerable amounts in the zona fasciculata, but all were scant to absent in the zona glomerulosa and zona reticularis. The Schiff reaction was strongly positive in the midzone of the fasciculata, diminishing in intensity toward the inner and outer portions. The Schiff reaction was negative in the reticularis and very weak in the glomerulosa. The brown pigment granules in the reticularis were both Schiff and Ashbel-Seligman negative. The medulla was not remarkable.

Thyroid: The symmetrically enlarged thyroid weighed 160 Gm. It contained multiple nodules, some partially calcified, measuring up to 4 cm. in diameter. On microscopic examination the gland was composed of large follicles filled with dense, nonvacuolated colloid and lined by flat epithelium. The nodules were poorly encapsulated. They were

composed of very small acini lined by cuboidal epithelium and embedded in a hyaline matrix. They were richly vascularized.

Two *parathyroid glands* and the *pancreas* were not remarkable. No gross *thymic* tissue was demonstrated.

Breast: Microscopic examination of the grossly enlarged breasts showed adipose tissue traversed by broad bands of almost acellular collagen within which were a moderate number of ducts. There was no heaping up of duct epithelium or evidence of secretory activity. Thus the gynecomastia of the gross examination appeared to be due to increased fat and connective tissue rather than to glandular activity or to a numerical increase of ducts.

Hypophysis: The hypophysis weighed 0.80 Gm. and was of normal configuration. It was sectioned in the median plane and stained by the periodic-acid Schiff (PAS) reaction, using an Orange G counterstain (6). The following cell types were classified:

1. *Normal basophils.* These are large, round or ovoid cells containing densely aggregated Schiff-positive granules. They occur chiefly around the periphery of the anterior lobe.

2. *Sparsely granulated Schiff-positive cells (amphophils).* These are rounded or polygonal cells which contain sparser, irregularly distributed granules which are Schiff-positive. By manipulation of pH and differentiation of a trichrome stain, the granules can be made to appear either blue or red (7). Some granules will also stain with acid hematein, as do the acidophils.

3. *Hypertrophic amphophils.* These cells are characterized by giant nuclei which are frequently bilobed or of irregular shape and may contain a prominent nucleolus. They are clearly derived from the sparsely granulated Schiff-positive cells through enlargement of the nucleus, diminution of the cytoplasm, and total degranulation (8).

4. *Chromophobes.* All agranular cells with small or moderate sized nuclei are classified as chromophobes.

5. *Acidophils.* These are rounded cells. Their densely packed granules stain with Orange G but are Schiff-negative.

A differential count on every fifth field of three widely spaced sections showed the following (Table 1): The normal basophils and acidophils were within normal limits. The sparsely granulated amphophils were increased significantly. The hypertrophic amphophils were also increased. The decrease in chromophobes was probably a reflec-

TABLE 1. HYPOPHYSIS: DIFFERENTIAL CELL COUNT
(Periodic-acid Schiff—Orange G technique)

Cell type	No. of cells counted	%	Av. % in 12 normal men aged 50 to 81 (Mellgren (8))
Normal basophils	323	10.2	12.2±1.86
Sparsely granulated amphophils	556	17.7	4.5±0.58
Hypertrophic amphophils	29	0.9	0.3±0.03
Acidophils	1,053	33.4	31.7±1.74
Chromophobes	1,198	37.8	51.3±1.58
Total	*3,159*	*100.0*	

tion of the relative increase in sparsely granulated cells. Two of 323 basophils examined showed Crooke's hyaline change (9).

The differential count is compared with average values for normal men obtained by Mellgren (8) in material stained with hematoxylin and basic fuchsin. This comparison with data obtained by a different method appears justified, inasmuch as a previous study has shown that results obtained with this technique are comparable with those found using PAS (10).

DISCUSSION

The techniques used in our work for the histologic study of lipoids in the testis and adrenal have been discussed elsewhere (5, 11, 12). The specificity of all these reactions, particularly the ones which purport to demonstrate steroids and ketonic carbonyl groups, have been questioned by many investigators (13). The reactions utilized here have been chosen so that to some extent one acts as a check on the other.

The distribution of lipoidal substances in the human testis has been studied by a number of investigators using different techniques (2, 14–20). The results indicate that in the normal mature male the interstitial cells, Sertoli cells, and germinal epithelium contain lipoid material and that in old age there is a general reduction of interstitial cell lipid (15, 19). Evidence has been accumulated to suggest a relationship between the lipid droplets in the interstitial cells and the production of androgenic steroids by the testis (14, 16, 21, 22). The findings in our case are thus compatible with, but not proof of, a reduced production of testicular androgens.

Human adrenals have likewise been studied histochemically by several investigators (8, 23–25). The results are not comparable, because different techniques were used and the cases studied were selected ones. At present no histochemical pattern of the adrenal can be defined as "normal," except within the grossest limits. The distribution of lipoids in the adrenal cortex in the case under discussion did not differ significantly from that in a group of cases without known endocrine stigmata now being studied in this laboratory.

The evaluation of human hypophyseal cytology in terms of function is difficult, as the sources of the various tropic hormones are not certainly known. As both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) contain glycoproteins, the Schiff-positive cells have been proposed as a likely source of these hormones (6). The high percentage of the sparsely granulated Schiff-positive amphophils found in this patient suggests that these cells may have been the source of his elevated urinary gonadotropins.

A hyperplasia of similar sparsely granulated Schiff-positive cells has been reported in a case of thyroid agenesis in which evidence was advanced that these cells may also, on occasion, produce thyrotropic hormone and some

TABLE 2. SUMMARY OF ANATOMIC FINDINGS IN CASES OF MALE HYPOGONADISM WITH TUBULAR ATROPHY AND PERSISTENT LEYDIG CELLS

Reference	Age (yrs.)	Ht. (cm.)	Build	Hair			Gynecomastia	Thyroid Wt. (Gm.)	Adrenal Wt. (Gm.)	Pituitary Wt. (Gm.)	Remarks
				Face	Ax.	Pub.					
(27)	59	163	Eun.*	0	0	0	0	15.5	6.1	*large*	Peptic ulcer, peritonitis
(28) #4	27	175	Eun.	0	0	sp**	+†	*large struma*	*very large*	0.64	Pulm. tuberculosis
(28) #5	55	176	Eun.	0	0	sp	0	*100*	normal	0.60	Pulm. tuberculosis
(28) #6	61	188	Eun.	0	0	sp	0	*microfoll. adenomas*	*1–2 cm. adenomas*	*11×12×9 mm.*	Carcinoma of bladder
(28) #7	54	177	Eun.	sp	0	0	—	*95 microfoll. adenomas*	*18*	*0.85*	Pulm. tuberculosis
(28) #8	55	165	Kyph.‡	sp	0	fem.	+	*45 adenomas*	15.2	*0.87*	Osseous tuberculosis, transverse myelitis
(29)	56	—	—	—	—	—	+	small	N	N	Dystrophia myotonica, elevated gonadotropins, BMR—18%
(28) #9	63	160	Fem.; fat	sp	—	0	+	25	N	0.62	Bronchitis, emphysema, cor pulmonale
(30)	55	158	Fem.; fat	0	sp	sp	+	small	small adenomas	*0.71*	Gastric ulcer, bronchopneumonia
(8) 030/38	55	—	Fem.; fat	0	—	fem.	—	—	*large adenoma*	—	—
(31)	50	—	Eun.; fat	sp	sp	sp	+	20 *small adenomas*	16	*0.85*	Hydrocephalus, bronchiectasis, osteoporosis
(28) #10	44	175	N§	sp	N	fem.	0	18.2	8.7	*0.90*	Burns with sepsis, lues, gonorrhea
(28) #11	48	161	N	sp	0	sp	0	18	10.8	0.65	Miliary tuberculosis
(28) #13	77	178	N	N	N	N	0	N	*17.3*	*1.12*	Emphysema, cor pulmonale
(32)	54	—	N	N	N	N	0	N	*large adenoma*	—	Dementia precox
(33) #1	40	162	N	N	N	N	0	22	16	0.68	Ruptured aorta
(33) #2	38	176	N	N	N	N	0	13	10	0.61	Miliary tuberculosis
(33) #3	44	167	N	N	N	N	0	20	16	*0.81*	Cerebral atrophy, phlegmon
(33) #5	64	164	N	N	N	N	0	22	*30*	*0.75*	Carcinoma of lung
(33) #6	70	165	N	N	N	N	0	*37*	*20*	0.60	Cholecystitis, pancreatitis, peritonitis
(33) #7	68	166	N	N	N	N	0	24	16	*0.70*	Multiple myeloma, sepsis
Our case, W.W.	63	170	Eun.	—	sp	fem.	+	*160 microfoll. adenomas*	N	*0.80*	Cerebrovascular accident; elevated gonadotropins; normal results with thyroid function tests

* Eun. = Eunuchoid.
** sp = sparse.
† + = present.
‡ Kyph. = Kyphotic.
§ N = normal.

sort of adrenocortical stimulating factor (26). If this were the case, abnormalities of the thyroid and adrenals might be expected to occur with some frequency in association with Klinefelter's syndrome.

Twenty-one cases of hypogonadism compatible with Klinefelter's syndrome were found in the older literature, and are summarized in Table 2. All these cases were characterized by testicular atrophy with azoospermia, partial or complete hyalinization of testicular tubules, and persistent Leydig cells which varied in number and morphology. Some patients had only a few atypical interstitial cells; others had masses of cells strongly suggestive of hyperplasia. The development of secondary sex characteristics varied. Six men (28 per cent) had large breasts which were not correlated with any constant morphologic change in either the Leydig cells or the adrenals. Six men (28 per cent) had large thyroids, 3 of which contained microfollicular adenomas. One other patient had a nodular thyroid of normal weight (31). Eight men (38 per cent) had grossly hyperplastic adrenals, 3 of which showed cortical nodules over 1 cm. in diameter. This is a high percentage, considering that in a series of 9,000 routine autopsies, the incidence of benign adrenocortical adenomas has been reported to be only 1.45 per cent (34). Therefore thyroid and adrenal changes are not constant, but the high incidence of hyperplasia of these glands suggests that they also participate in the hormonal balance of Klinefelter's syndrome.

SUMMARY

A 63-year-old man with Klinefelter's syndrome, married but childless, exhibited moderately eunuchoid habitus, large breasts, nodular goiter and small testes. Urinary gonadotropin excretion was elevated and thyroid function tests gave normal results. At autopsy, the testes were composed chiefly of massed interstitial cells, and the findings on histochemical examination were compatible with a reduced production of androgenic steroids. Tubules were exceedingly rare and totally hyalinized; a few still contained Sertoli cells. There was a multinodular goiter weighing 160 Gm. The adrenals were not remarkable on routine and histochemical microscopic examination. The hypophysis was enlarged (0.80 Gm.). Sparsely granulated Schiff-positive cells and hypertrophic nongranulated cells derived from them were significantly increased in number. Basophils and acidophils were present in normal proportions.

A review of 21 cases from the literature of male hypogonadism showed a high incidence of thyroid or adrenal hyperplasia. It is suggested that these organs may participate in the endocrine imbalance of Klinefelter's syndrome.

Acknowledgments

Dr. Samuel L. Gargill contributed many of the clinical data. Dr. Felix G. Fleischner reviewed the radiograms of this case. Technical assistance was rendered by Miss Theresa Heneghan and Miss Doris Parthum.

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CHANGES IN HUMAN PLASMA MUCOPROTEINS IN HYPERTHYROIDISM AND MYXEDEMA*

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ELECTROPHORETIC (1–4) or chemical (5–8) determinations of the circulating mucoproteins in man indicate that their level is frequently elevated in pathologic conditions such as cancer, pneumonia or rheumatoid arthritis. The more normal values found in treated patients (1, 4, 8) suggest that a relationship exists between the activity of the disease process and the level of circulating mucoproteins. It was of interest, therefore, to study the level of the circulating mucoproteins in a disease process in which the course could be reversed with the appropriate therapeutic measures. The syndromes of hyperthyroidism and myxedema fulfill this criterion. In addition, they represent to a large extent the effects of the extremes in function of the same endocrine gland.

The plasma levels of the mucoproteins M-1 and M-2 have therefore been determined by electrophoretic analysis at pH 4.5 (2) in hyperthyroid and in myxedematous patients. The plasmas were then re-examined after the patients had been brought to a euthyroid state.

SUBJECTS AND METHODS

Subjects

Four patients with frank hyperthyroidism and 6 with myxedema were the subjects of this study. They all had an extreme degree of abnormal thyroid function as shown by the classic clinical signs and the findings in the indicated laboratory tests. These tests included some or all of the following: BMR, concentration of serum cholesterol and protein-bound iodine, and I^{131} uptake and excretion studies. The uptake and excretion of I^{131} was measured in 3 of the 4 patients with hyperthyroidism, and in each instance over 60 per cent of a tracer dose was concentrated in the neck at forty-eight hours. Similar studies were made in all of the patients with

Received for publication November 13, 1953.

* The authors wish to acknowledge the assistance of the Damon Runyon Memorial Fund, the Atomic Energy Commission (Contract No. AT(30-1)-910), and the National Cancer Institute of the United States Public Health Service (Contract No. C-471).

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myxedema. In these patients less than 5 per cent of the dose was concentrated in the neck.

Thyrotoxic patients were brought to a euthyroid state with one of the thiourea derivatives (usually propylthiouracil), or with a therapeutic dose of I^{131}. Myxedematous patients were treated with desiccated thyroid in doses necessary to bring them to a euthyroid state.

Criteria for euthyroidism included a reversion to normal of the physical and laboratory findings just mentioned with the exception of the I^{131} uptake values, which could not be expected to revert to normal in either myxedematous patients or in thyrotoxic patients treated with antithyroid drugs.

Electrophoretic analyses

Blood was drawn from patients in the postabsorptive state and was prevented from clotting by the addition of one-tenth volume of 5 per cent sodium citrate. The total plasma proteins were determined by the biuret method, and corrected for the dilution (9). The plasma was mixed with two volumes of 0.15 M sodium chloride, to give a 2.0–2.5 per cent protein solution, and dialyzed for two days at 5° C. against two lots of a buffer of 0.1 ionic strength at pH 4.5 (0.08 M sodium chloride and 0.02 M sodium acetate, with the pH adjusted with acetic acid (10)).

Prior to analysis, the dialyzed sample was centrifuged to remove a small amount of precipitate. To obviate the difficulties associated with a lengthy compensation, an 11.0 ml. short cell was used, with the starting boundaries behind the center plates. Electrophoresis was carried out at 0.2° C. with a potential gradient of 5.6 volts per cm. Scanning photographs were taken with a gear ratio of 4 to 1 at the beginning of the experiment and 210 minutes later. The boundaries were then moved up about 1 centimeter, by compensating, to bring into view the portion of the pattern hidden by the ground-glass plates, and an additional set of photographs taken. The patterns in which the mucroprotein boundaries had ascended were enlarged and traced on graph paper over the corresponding baselines. In order to resolve the M-2 boundary from the overlapping albumin boundary, a tracing of an albumin boundary[1] was used (Fig. 1). No attempt was made to resolve the albumin and globulin boundaries, and no correction was made for the salt boundary, which was included in the total area.

RESULTS

Representative patterns obtained on the two groups of patients are shown in Figure 1. The most consistent variations appeared in the region of mucoprotein M-2, where the plasmas of thyrotoxic patients showed a definitely larger area when compared with those of the myxedematous patients. The values obtained for M-2 and M-1 are given in Table 1. Although there was considerable scatter in the individual M-2 values, the average

[1] A 1 per cent solution of human plasma albumin was analyzed electrophoretically under the same conditions as were used in the plasma experiments. The albumin was obtained through the courtesy of Dr. S. T. Gibson of the American Red Cross and Dr. J. N. Ashworth of E. R. Squibb and Sons.

TABLE 1

Case No.	Status	Total protein (Gm./100 ml.)	M-2 (mg./100 ml.)	M-1 (mg./100 ml.)
1	Hyperthyroid	6.9	270	40
	Euthyroid	6.8	180	30
2	Hyperthyroid	6.8	310	60
	Euthyroid	7.1	280	40
3	Hyperthyroid	7.2	380	80
	Euthyroid	7.6	310	40
4	Hyperthyroid	5.9	220	50
	Euthyroid	6.6	220	50
av.	*Hyperthyroid*	*6.7*	*300*	*58*
	Euthyroid	*7.0*	*248*	*40*
5	Myxedematous	7.6	180	10
	Euthyroid	8.2	200	40
6	Myxedematous	6.3	120	30
	Euthyroid	6.3	250	60
7	Myxedematous	7.2	250	50
	Euthyroid	5.9	320	90
8	Myxedematous	7.6	180	80
	Euthyroid	6.8	270	80
9	Myxedematous	7.9	200	100
	Euthyroid	7.2	260	50
10	Myxedematous	6.9	220	20
	Euthyroid	6.3	200	30
av.	*Myxedematous*	*7.3*	*190*	*48*
	Euthyroid	*6.8*	*250*	*60*

for the hyperthyroid group was 300 mg. per 100 ml., considerably higher than the average for the myxedematous group (190 mg. per 100 ml.). After treatment, both groups had the same average M-2 values, namely, 250 mg. per 100 ml.

In the hyperthyroid group, 3 of 4 patients showed decreases in the concentration of M-2 following treatment. The reverse change was produced in 5 of 6 myxedematous patients following therapy.

Although the amounts of M-1 present were so small that their determination was subject to considerable experimental error, the changes in M-1 appeared to run parallel to changes in M-2 in most of the cases studied.

The average concentration of the total plasma proteins appeared to be elevated in the patients with myxedema and to fall upon treatment with thyroid. The concentration of the total plasma proteins in the patients with hyperthyroidism, on the other hand, was low when their disease was active and rose upon reversion to a euthyroid state.

Since the change in concentrations of M-2 and M-1 was in the reverse

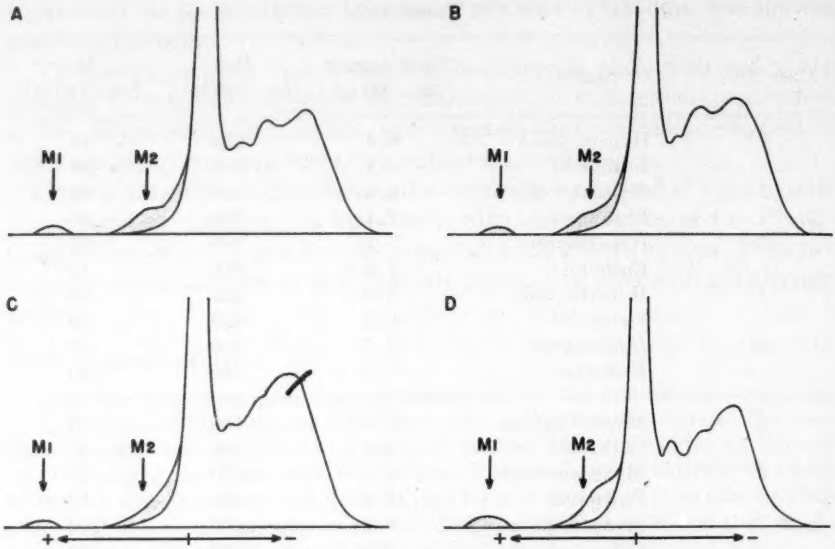

FIG. 1. Electrophoretic patterns of plasma at pH 4.5. **A.** Hyperthyroid patient (Case 2). **B.** Same patient, euthyroid. **C.** Myxedematous patient (Case 7). **D.** Same patient, euthyroid.

direction from the change in concentration of total plasma proteins, the significance of the changes in the mucoproteins is increased.

DISCUSSION

The nature and physiologic significance of the plasma mucoproteins are still not well understood (11). One protein with the electrophoretic properties of M-1 has been isolated and characterized (12, 13). It has a molecular weight of 44,100 (14), and an isoelectric point near pH 2.0 (12, 13). In the conventional electrophoretic analysis at pH 8.6 it is included with the alpha-1 fraction (12). It is rich in hexose and hexosamine (12, 13). Its reaction with collagen *in vitro* (15) suggests that it may be a normal component of connective tissue, although substances other than this mucoprotein have similar effects on collagen (16).

Even less is known about the mucoproteins which make up component M-2. One of them appears to correspond to the alpha-2 glycoprotein recently described by Schmid (17), which has an isoelectric point between pH 4.1 and 4.3 and a sedimentation constant of 3.0 S. Electrophoretic separation experiments at pH 4.5 on plasmas containing I^{131}-thyroxine are now being carried out in this laboratory.[2] If the thyroxine-binding protein

[2] Note added in proof: *See* Robbins, J.; Petermann, M. L., and Rall, J. E.: *Fed. Proc.* **13**: 280, 1954.

(18) were included in the proteins measured as M-2, changes in this protein which correlate positively with changes in concentration of circulating thyroxine would present an interesting and probably significant finding.

The source of the circulating M-2 is still unknown. Since it increases in thyroidectomized patients when their myxedema is corrected by exogenous thyroid, it apparently does not originate in the thyroid gland. Furthermore, 2 patients with Graves' disease showed no change in either M-2 or M-1 after treatment with amounts of thyroid-stimulating hormone which caused marked increases in thyroidal activity as measured by secretion of labeled thyroid hormone by the thyroid.

Recent studies have indicated that in severe myxedema the serum mucoproteins determined by chemical methods are elevated over the normal values (19). The discrepancy between these findings and the low values reported in this paper cannot be explained until more information is available about the various mucoproteins of plasma and the correlation between the chemical and electrophoretic methods of measuring them.

The increase in concentrations of total proteins in plasma in patients with myxedema noted in this study has been reported previously (20). A reciprocal decrease in concentration of plasma proteins was found in hyperthyroidism.

SUMMARY

1. The plasma mucoprotein components M-1 and M-2 have been measured in patients with thyroid dysfunction by electrophoretic analysis at pH 4.5.

2. Thyrotoxic patients displayed elevated levels of M-2, which declined when they became euthyroid.

3. Myxedematous patients had low levels of M-2, which increased when they became euthyroid.

4. Changes in M-1 were generally parallel to those found for M-2.

5. The concentrations of the total plasma proteins generally rose with decreasing thyroidal activity and fell with increasing thyroidal activity.

Acknowledgment

The authors wish to thank Dr. Rulon W. Rawson for advice and encouragement, and Mrs. Mary G. Hamilton for assistance with the electrophoretic analyses.

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REPORT OF A PATIENT WITH TYPICAL PROGERIA, INCLUDING DATA FROM URINARY HORMONE STUDIES*

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THE syndrome of true progeria, first described by Hutchinson (1) in 1886 and further classified by Gilford (2) in 1904, has been reported since that time only on rare occasions. A recent review of the world literature by Thomson and Forfar(3) lists only 19 reports of typical progeria, including their own patient. Wilkins (4) has recently described another patient.

The diagnosis is based on the bizarre appearance of the patient and thus far no definitive biochemical laboratory findings have been established. The description of our patient, W. R., and his photographs are typical of classic progeria. The long period of his followup and the data of his urinary hormone excretion appeared to warrant this report.

The necropsy findings reported by Gilford (2), Orrico and Strada (5) and Talbot *et al.* (6) give supportive evidence to the clinical impression of premature aging. These findings include advanced arteriosclerotic changes, atherosclerosis of the aorta, narrowing of the coronary arteries and calcareous deposits in the heart valves.

Roentgenologic studies reportedly show an advancement of osseous age (6, 7). A tendency towards narrowing of the shafts of long bones when compared to epiphyses is noted and the femoral neck tends to continue in a straight line with the axis of the femoral shaft (6, 7).

Talbot *et al.* (6) made a detailed metabolic balance study on a progeric patient aged 6½ years. By choice, the patient ingested a diet supplying about 103 calories per kilo of weight, which was equivalent to the caloric intake of a normal 1-year-old child of the same weight. Balance studies re-

Received for publication November 19, 1953.

* This study was aided by grants to one of us (E.C.H.) from the Research Council of Duke University, from Ayerst, McKenna and Harrison, Ltd., New York, N. Y., and from Schering Corporation, Bloomfield, N. J.

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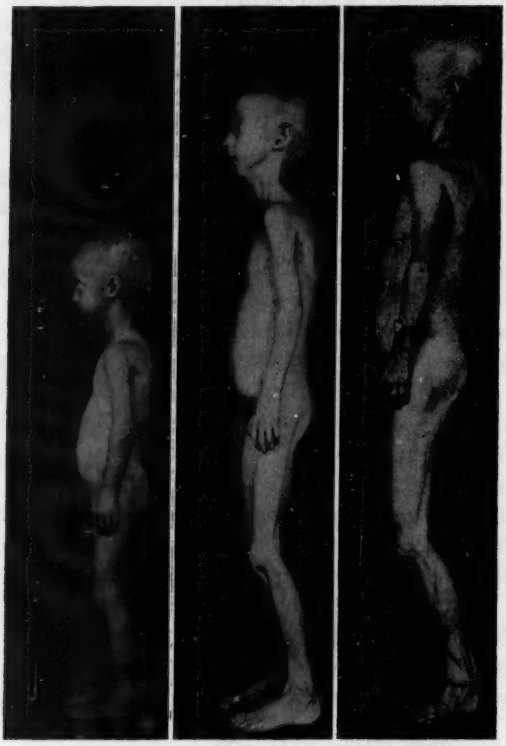
vealed that the progeric patient utilized about 95 calories per kilo for basal energy requirement, leaving very little for growth and further development. This hypermetabolism was reflected in an elevated basal metabolic rate and tachycardia. There was no other evidence of hyperthyroidism and the serum protein-bound iodine level was normal. This study, therefore, suggested a basic metabolic error which may supply the key to the etiology of progeria.

CASE REPORT

W. R., a white male, first came under the observation of one of us (ECH) in June 1937 when he was $4\frac{10}{12}$ years of age (Fig. 1A) because of anorexia, weakness, failure to gain weight normally and loss of hair since the age of one year.



A B C

FIG. 1. Lateral photographs of patient taken to the same scale. These were made at ages $4\frac{10}{12}$, $7\frac{11}{12}$, and $19\frac{0}{12}$ years of age, left to right, respectively.

As far as is known, he was born normally and spontaneously at full term (weighing 7 pounds, 12 ounces) following a normal pregnancy. He was breast fed for eight months

and then given a formula diet. Severe diarrhea began at the age of 9 months and continued until he was about 12 months old, in spite of several changes of formula.

The patient was able to hold up his head at 4 months, walk at 12 to 14 months and form sentences at about 24 months. Weakness and failure to gain weight adequately were noticed onward from the age of 4 months. When he was 12 months old he weighed only 13 pounds, the scalp hair began to fall out and he was almost bald at 2 years, at which time he weighed 19 pounds. Primary dentition began at 12 months and was complete at $4\frac{1}{2}$ years.

At age $4\frac{11}{12}$, in spite of obviously marked underdevelopment, he was active and ran around considerably. He had an aged, wizened appearance with small facial features and a disproportionately large head. The scalp was dry, lifeless and parchment-like with prominent veins. Almost no hair remained. The fontanelles were closed and prominent frontal bossing was noted.

The skin was dry, inelastic and wrinkled, without unusual pigmentation. The heart and lungs were essentially normal upon physical examination. The thyroid was not palpably enlarged. The abdomen was rather protuberant and the liver was slightly enlarged to percussion and palpation.

The genitalia were well formed, with a normal sized penis. The testes were of average size and normally situated in a well formed scrotum.

At the time of observation the temperature was 37.7 degrees C., pulse 124, and respirations 28. The anthropometric measurements are contained in Table 1. The report of x-ray examination of the skull described the sella turcica as normal, and the bone age was advanced to about 7 years.

Table 1. Anthropometric measurements of Patient W.R.

	Age $4\frac{11}{12}$ yrs.	Age $19\frac{0}{12}$ yrs.	Min. normal measurements for age 19 yrs.
Height (inches)	36.5	50.75	65.3
Weight (pounds)	22	46	117.2
Span (inches)	34	43	67.2
Upper measurement (head to pubis) (inches)	20	25.5	31.9
Lower measurement (pubis to floor) (inches)	16.5	25.25	32.6
Head (circumference) (inches)	19	19.25	22.5
Chest (inches)	19	23.5	34.4
Waist (inches)	18	24.5	28.5
Hips (inches)	16.5	24.25	

Laboratory findings were as follows: hemoglobin 77 per cent; red cell count 3,950,000; white cell count 10,600; differential white cell count—PMN 71 per cent, LLM 0 per cent, SLM 24 per cent, Tr. 3 per cent, Eos. 1 per cent, B. 1 per cent; cell morphology normal; urine normal, with a specific gravity of 1.010. X-ray examination of the skull did not reveal any evidence of pituitary tumor.

The administration of whole anterior pituitary extract ("Polyansyn," Ayerst, Mc-

Kenna and Harrison) was tried for several weeks and though an initial general improvement and a weight gain of 4½ pounds were noted, these were not sustained and the therapy was abandoned.

In July 1939 (age 6$\frac{11}{12}$ years) his mother reported by letter that his height was now 40½ inches and weight 25 pounds. He had survived whooping cough and measles and was now preparing to begin school.

He was next seen one year later at the age of 7$\frac{11}{12}$ years (Figs. 1B and 2). He had had nausea and vomiting for one week. Upon admission, the temperature was 37.7 degrees C., pulse 142, respirations 40, and blood pressure 80/40. The nasopharynx was markedly injected and a diagnosis of upper respiratory infection was made.

FIG. 2. Hands, showing senile appearance and trophic changes of the nails at age of 7$\frac{11}{12}$ years.

On admission, urinalysis showed a trace of sugar and 4 plus acetonuria. The red cell count was 5,250,000, hemoglobin 103 per cent and white cell count 14,360. Examination of the stools showed no blood and parasites. The serum showed the following concentrations: NPN 32 mg., calcium 12.9 mg., phosphorus 3.8 mg., and cholesterol 218 mg. per 100 ml.

Following administration of intravenous fluids, the patient quickly improved and was discharged five days after admission.

At age 13 to 14, he began having erections and nocturnal emissions and had begun to develop a few coarse facial hairs at age 17.

The patient was not seen again until August 1951, at the age of 19$\frac{0}{12}$ years (Fig. 1C). During the 11-year interval he had been generally well, had shown remarkable social

adjustment and had graduated second in his high school class. In spite of his physical handicap, he could type 37 words per minute.

His temperature was 37 degrees C., pulse 72, respirations 16, and blood pressure 114/80. There was very scant, straggling scalp hair. Pubic hair was present, but also scant. No other body hair was noted.

The skin had a generalized brownish mottled pigmentation and the hands were dry, hard and scaly. The nails were atrophic. The eyes were prominent, but essentially normal pupillary responses and extraocular movements were noted. Ophthalmologic examination revealed normal discs with minimal tortuosity of the smaller vessels.

The teeth were irregular and crowded close together. The palatal arch was high. The thyroid was not enlarged to palpation and the trachea was midline in position.

The thorax was narrow but the lungs were normal to auscultation and percussion. A systolic bruit was audible over the entire precordium, but most marked in the mitral area. The abdomen was relatively protuberant but otherwise normal.

The patient stood in a typical slightly crouched position at all times. Tendon reflexes were generally hypoactive, but good strength for muscle mass was noted. The anthropometric measurements are contained in Table 1.

The penis was 2 inches in length and erectile. Both testes were normally situated in a normal scrotum. They measured about 1.75 inches by 0.75 inches. Rectal examination revealed a well developed prostate, which was normal to palpation.

Laboratory studies were very limited in scope because the patient would not cooperate. The urine was normal, hemoglobin 100 per cent, and red blood cell count 3,870,000. The serum concentration of sodium was 150 mEq. per liter, of potassium 4.1 mEq. per liter, and of cholesterol 180 mg. per 100 ml. The basal metabolic rate was reported as +40 per cent by the DuBois method and +73 per cent by the Lewis method, but the test was considered of doubtful value.

The urinary gonadotropins, using the method described by Easterling *et al.* (11), were reported as 3 rat uterine units per twenty-four hours. The range of normal for adult males aged 20 to 45 years, by this method, is 2 to 8 rat uterine units per twenty-four hours. The urinary 17-ketosteroids were 4.5 mg. per twenty-four hours. Our laboratory considers the range of normal for urinary 17-ketosteroids for adult healthy males aged 20 to 45 years to be 10 to 22 milligrams per twenty-four hours.

The patient is at present (October 1953) $21\frac{2}{12}$ years of age and as far as is known is still in good health. He operates a small grocery business and is evidently successful in this venture.

DISCUSSION

The etiology of progeria remains unknown; several authors have suggested that it is of pituitary origin. The contrast between acromegaly and progeria, first indicated by Gilford (2), and some points of similarity with Simmonds' disease (3) have been cited as evidence for this hypothesis. Abnormalities of the sella turcica have been described by Thomson and Forfar (3), Zeder (9), and Eschaquet (10). Orrico's (5) patient exhibited a cystic abnormality in the region of the pars intermedia and marked hypoplasia of the adrenal cortex was described. The level of urinary excretion of gonadotropins by our patient does not suggest hypopituitarism.

Information concerning endocrine function is very limited; nevertheless,

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Information concerning endocrine function is very limited; nevertheless,

some conclusions may be drawn. Adrenocortical insufficiency is not suggested by the normal or slightly elevated blood pressures and normal blood glucose values. The blood electrolyte studies reported by Talbot (6), and those of our own patient are within the normal range. The dextrose tolerance curve of Talbot's patient was also normal. The urinary 17-ketosteroid excretion has been reported at the upper border of normal (3). The excretion of W. R. was below the normal range, but this may be related, certainly in part, to his understature and his subnormal weight.

The tendency toward normal or above-average intelligence (2, 3, 6, 8), advancement of bone age and the normal serum protein-bound iodine level (6) certainly do not suggest hypothyroidism.

In two reports (5, 6) no evidence of spermatogenesis was found at postmortem examination; on the other hand, Gilford (2) described spermatogenesis in 1 patient. The urinary excretion of gonadotropins by our patient was normal and was compatible with spermatogenesis. A seminal examination could not be secured.

On the basis of such inadequate information, it is not possible to rule out an endocrine deficiency, but it would appear much more plausible that the basic etiology is a genetic error in general cellular metabolism. Such disordered metabolism could account for the evidences of endocrine deficiency thus far described.

SUMMARY

A brief review of the literature and the case history of a male patient with typical progeria are presented. His level of excretion of urinary gonadotropins was normal, whereas that of urinary 17-ketosteroids was low, when judged by standards for a healthy adult male of normal height and weight. However, there was no conclusive evidence of hypogonadism.

A discussion of progeria and its relationship to the endocrine glands is presented.

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CAT-SCRATCH THYROIDITIS TREATED WITH THYROTROPIC HORMONE

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with the technical assistance of BARBARA ERSKINE
The Paoli Medical Center, Paoli, Pennsylvania

CAT-SCRATCH disease is a lympho-reticuloendotheliosis related to the lymphogranuloma-psitticine group. It has been responsible for protean inflammation of the central nervous system, eye, skin and lymph nodes. This case is reported 1) because we believe that it is the first instance of severe acute thyroiditis caused by the virus of cat-scratch disease, and 2) because the use of thyrotropic hormone brought about prompt cessation of the disease. ACTH, TSH and cortisone have been used successfully in the treatment of acute thyroiditis (1) (2).

CASE REPORT

A 13-year-old white girl was admitted to the Davis Clinic, Paoli Medical Center, June 6, 1953, with a hard, painful mass in the left side of her neck. The mass was the exact shape of the thyroid lobe, only about ten times normal size. There were many scratch marks over her forearms, arms and chest, and a vaccination-like area, 2.5 cm. in diameter to the left of the sternoclavicular junction (Fig. 1). One week prior to admission she had

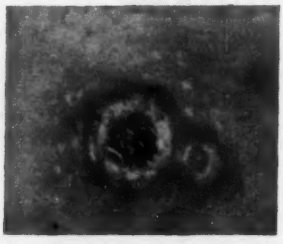

FIGURE 1

had chills, fever, fatigue, dysphagia, cough, a localized eruption on her chest and pain in her left ear. Treatment elsewhere with all sorts of antibiotics had produced no apparent improvement. The fever, dysphagia, pain and swelling increased. Her temperature was 104.8° F. orally, her pulse rate 96, and her respirations 28 per minute. Examination revealed scratch marks on the forearms, arms, and chest and a large vaccination-like mark below the left clavicle. The left lobe of the thyroid gland was ten times the normal size, hard and tender. A roentgenogram of the chest, urinalysis, red cell counts, hemoglobin

Received for publication October 24, 1953.

determination and differential counts showed nothing remarkable. The total white cell count ranged from 8,900 to 10,000 per cubic millimeter. The Wintrobe sedimentation rate was 42. Blood cultures were sterile. Serologic studies carried out through the courtesy of the Virus Laboratory of the Children's Hospital of Philadelphia gave negative results for the lymphogranuloma-psitticine group of diseases. Heterophile agglutination tests at intervals gave negative reactions, as did the Frei test. The child stated she had been playing with a mother cat and her six kittens for many weeks. As the cats grew larger they began to claw her when she fondled them.

The fever and the tenderness of the thyroid gland continued unabated, in spite of intensive antibiotic therapy elsewhere. Since these treatments had been unsuccessful and there was no evidence of suppuration, we decided to institute thyrotropic hormone therapy. Accordingly, Thytropar[1] was started intramuscualrly in a dosage of 10 units daily, for four days. The following day the temperature fell from 104.8° F., where it had been for over a week, to 100° F. The next day the temperature became normal. The tenderness and pain subsided and the patient appeared improved and stated that she felt much better. The fifth day she was given 20 mg. of ACTH gel, which was continued for the next five days; she then received 10 mg. per day for the next five days, after which it was discontinued. A week later the thyroid gland had returned to approximately normal size.

DISCUSSION

In this patient the concept of virus etiology in acute thyroiditis is suggested because of the visible cat scratches, the evident vaccination-like lesion and the failure of antibiotics.

The prompt disappearance of fever and symptoms suggest the beneficial effect of TSH in the therapy of acute thyroiditis.

SUMMARY

A case is reported of acute thyroiditis caused by cat scratch in a young female who responded favorably to the administration of TSH.

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[1] Graciously supplied by the Armour Laboratories, Chicago, Illinois.

Letters to the Editor

ASCORBIC ACID AND URINARY STEROID EXCRETION

TO THE EDITOR:

The recent report by Klein, Papadatos and Fortunato (1) in the January 1954 issue of this *Journal* interests us.[1] These authors reported that administration of ascorbic acid to normal human subjects resulted in a marked decrease in the urinary substances giving the "blue chromogen" reaction, but failed to alter the urinary content of 17-ketosteroids (17-KS). Such findings are reminiscent of data collected in this laboratory some time ago. We observed that in adrenalectomized female rats given cortisone, administration of ascorbic acid prevented the rise in urinary 17-KS observed when ascorbic acid was not administered (2). Further work indicated that ascorbic-acid treatment failed to alter the increased urinary 17-KS resulting from the injection of dehydroepiandrosterone into adrenalectomized female rats (3). These data indicated that ascorbic acid exerted its action, not on the endogenous 17-KS, or on secreted 17-KS, but rather on the formation of 17-KS from the injected cortisone. This aspect of the action of ascorbic acid has been shown by numerous *in vitro* experiments (4). It was also found that the ascorbic-acid treatment of intact female rats resulted in only a slight decrease in the urinary 17-KS.

The failure to observe any marked change in the urinary 17-KS of intact subjects following ascorbic-acid treatment is probably due to the following factors: (a) in the intact organism much of the 17-KS excreted in the urine is derived from the testes and/or the ovaries and from the adrenal cortex, as secreted 17-KS; (b) in addition, some of the 17-ketosteroids are derived from the metabolism of C-21 corticosteroids from the adrenal. The fact that ascorbic acid failed to alter markedly the endogenous 17-KS in adrenalectomized and in intact rats, and in intact human subjects, suggests that this vitamin does not alter the metabolism of already-present (secreted) 17-KS, at least to the degree detectable by the Zimmermann reaction. Ascorbic acid was observed to decrease the *17-KS derivatives of injected cortisone* (2).

It is indeed fortunate that Klein *et al.* studied the alterations in the "blue chromogens" following ascorbic-acid treatment in intact subjects. These "blue chromogens," as well as the Pettenkofer-positive materials, constitute 20 to 30 per cent of the total 17-KS in the urine of normal sub-

[1] Klein *et al.* (*J. Clin. Endocrinol. & Metab.* **14:** 105 (Jan.) 1954) stated that we reported that "the administration of ascorbic acid to rats depresses the urinary excretion of 17-ketosteroids." This statement is an inaccurate representation of our data. The correct information, as evident from our papers (2), is that the administration of ascorbic acid to cortisone-treated adrenalectomized rats depresses the urinary levels of the 17-KS metabolites of the cortisone.

jects. Moreover, these substances, of which dehydroepiandrosterone is *a constituent*, are considered to be derivatives of certain C-21 corticosteroids (5), and are probably not dependent on the testes (6). Since these blue chromogens constitute such a relatively small component of the urinary 17-KS it is readily understood why marked changes (for example, a mean decrease of 52 per cent as found by Klein *et al.*) in the urinary content of these blue chromogens are reflected in the urinary 17-KS only to a very small extent. In view of the foregoing considerations, it is necessary to interpret the findings of Klein *et al.* as indicating that administration of ascorbic acid to intact subjects is capable of depressing the excretion of substances which include metabolites of certain adrenocortical hormones. Since the Pettenkofer reaction under these circumstances is known to measure dehydroepiandrosterone and some closely related steroids, it is difficult to indicate specifically which corticosteroids are involved.

Observations such as those presented further emphasize the importance of measures to eliminate or minimize the contribution of endogenous and of *secreted 17-KS* on results in studies of the *17-KS metabolites* of corticosteroids. In our experiments, adrenalectomized female rats were employed; these rats excrete minimal amounts of 17-KS. The great increase of urinary 17-KS following injection of cortisone or dehydroepiandrosterone into these animals was, therefore, reasonably ascribed to the metabolism of the injected steroids. The use of adrenalectomized preparations for experimental studies is advantageous, and in our studies an additional reason was to eliminate the interference of alterations of activity of the pituitary-adrenal axis.

It should be emphasized that the data on blue chromogens provided by Klein *et al.* are in essential agreement with the conclusions derived from our studies on the effect of the administration of ascorbic acid on the excretion of urinary metabolites of adrenal steroids.

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ESTIMATION OF 17-HYDROXYCORTICOIDS IN PERIPHERAL HUMAN BLOOD

To the Editor:

A modified method for the estimation of 17-hydroxycorticoids in peripheral blood has been developed. After extraction of a 20-ml. sample of plasma with chloroform, the extract is purified by dissolving it in 70 per cent methanol and partitioning between toluene-hexane and pentane.

After taking up the methanol dry residue in ethylacetate, this solution is washed with aqueous Na_2CO_3 and finally with 0.01N HCl. The ethylacetate solution is taken to dryness and in the residue the 17-hydroxycorticoids are measured by a modified Porter-Silber reaction, making a correction for background absorption by measuring before and after the addition of phenylhydrazine, after heating with sulfuric acid.

Normal plasma levels of 17-hydroxycorticoids obtained with this method are 6.3 ± 1.2 μg. per 100 ml. in males and 6.1 ± 1.3 μg. per 100 ml. in females. Patients suffering from Cushing's disease show values up to 20 μg. per 100 ml. After injection of ACTH, values up to 38 μg. per 100 ml. have been observed.

The time-response relationship was studied after injection of normal males with regular ACTH and with the long-acting product, ZnACTH (Cortrophine-Z®, Organon). The results indicate that the level of 17-hydroxycorticoids in the blood remains increased for at least twelve hours after injection of ZnACTH, whereas after injection of the regular product the blood level returns to its initial value within six hours.

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CRETINISM, WITH OR WITHOUT GOITER, IN 5 OF 10 SIBLINGS

CRETINISM develops before birth and, unless suspected, usually is not recognized until the child is about 9 months of age, at which time retarded physical development becomes evident. When cretinism occurs in endemic form there is usually enlargement of the thyroid gland. The goiter is often striking and shows various histologic changes, namely, nodules with areas of hyperplasia and involution. Cretinism may occur in endemic form without goiter, as demonstrated in the family to be discussed. In sporadic cretinism, thyroid agenesis is found. In most cases there is only a remnant of thyroid gland, usually manifest as undifferentiated lymphoid tissue.

In the family under discussion there were 5 cretins (Fig. 1 and Table 1), 3 with goiters, in a total of 10 children. Of the 5 noncretin children, 1 had a goiter with hyperthyroidism, and the other 4 were considered normal and free of thyroid disease. The mother and father of these children did not have goiter or any gross evidence of thyroid dysfunction. The family lived in Northern Oklahoma until the youngest of the cretins was aged 6, when they moved to a farm in Southern Missouri. The mother and father were second cousins. All of the children grew up under the same environment and none of them presented any serious feeding problem during their period of development. One maternal aunt may have had a goiter but this was not a certainty from the information obtained. The cretins were the third, fifth, sixth, eighth and ninth siblings of this family of ten.

CASE I

Georgia C. aged 33, was 8 years of age when a swelling in her neck was first noticed. This continued to enlarge and attained its present size when she was about 16 years old. She attended school for three years only, finding it difficult to learn. She always had difficulty in talking and her voice had always been husky. She began to menstruate at the age of 20; further menstrual history was unreliable. She lived with her family and was able to do small routine chores about the house.

Examination showed perception and response to be slow. The voice was guttural when she occasionally answered questions with monosyllables. The facies was cretinoid. Her weight was 80 pounds, height 52 inches, and pulse rate 108. Retarded growth of long bones gave the proportions of a child. The hair was fine in texture and the skin wrinkled and thick. The teeth were badly crowded with marked caries. The tongue was deeply furrowed. There was a large nodular goiter. A loud murmur was heard over the superior thyroid vessels, and the veins of the neck were dilated.

Laboratory studies: The blood count, urine, and concentrations of fasting blood sugar

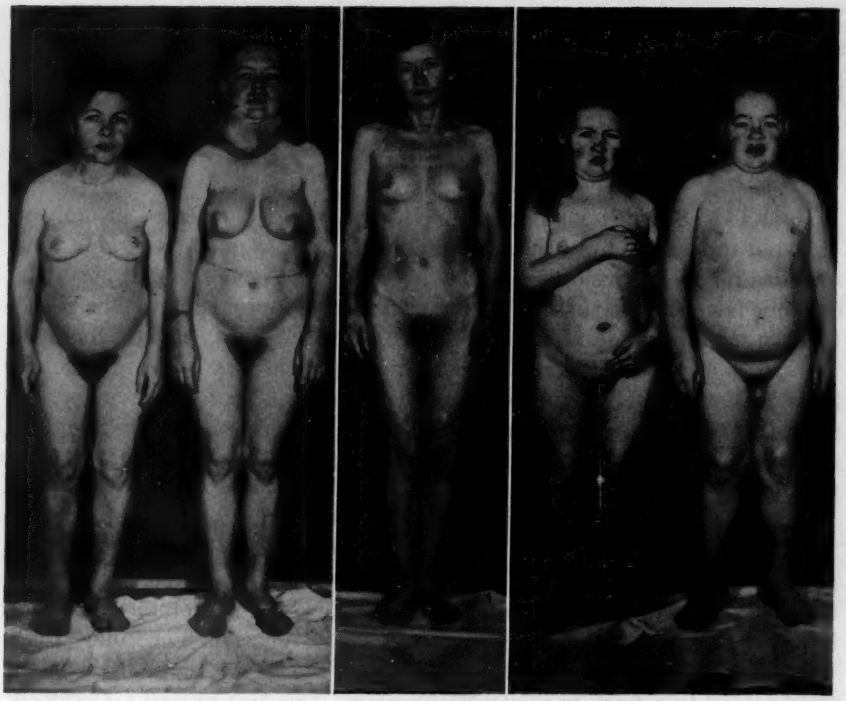

FIG. 1

Case No.	I	II	III	IV	V
Age (yrs.)	33	29	27	22	21
Ht. (ins.)	52	57	58	51	53

and nonprotein nitrogen were found to be normal. The serum cholesterol level was 118 mg. per 100 cc. Two basal metabolic rate determinations were +26 and +21 per cent. Liver function tests (cephalin flocculation and thymol turbidity) gave normal findings. The sugar tolerance curve was flat. X-ray examination of the long bones showed that the femoral heads were flattened and the necks were shortened. All bone centers were fused. A lateral skull x-ray film showed a proportionate increase in the antero-posterior diameter of the skull, a normal sella turcica, and hyperostoses frontalis interna. Fluoroscopic examination of the chest gave evidence of compression of the trachea in the thyroid area. Special ocular examination showed no variations in the ocular fundi. An electroencephalogram revealed altered cortical excitability. Following a tracer dose of radioactive iodine there was a urinary excretion of 47 per cent.

Progress: In November 1941 a total thyroidectomy was performed. The tissue removed weighed 725 Gm. Microscopic examination (Fig. 2) revealed many small thyroid acini, lined by cuboidal epithelium and distended with an abundant pink-staining colloid material. Several large cystic spaces, engorged with the same material were noted. There was a marked hyalinized fibrosis of the interstitial tissue, with lymphocytic infiltration. Many areas suggested the histologic pattern of "fetal adenoma."

TABLE 1

Case No., age and sex	Height (inches)	Goiter	Average BMR (%)	Blood cholesterol (mg. /100 cc.)	I^{131} excretion (%)	Menses; age of patient at onset
I 33 yrs., F	52	Yes	+21	280	47.0	Yes; 20 yrs.
II 29 yrs., F	57	Yes	+35	178	16.5	Yes, ?
III 27 yrs., F	58	Yes (2 operations)	+ 3 (one test)	443	—	Yes; 16 yrs.
IV 22 yrs., F	51	0	− 5 (one test)	390	58.0	Yes; 17 yrs.
V 21 yrs., M	53	0	− 9 (one test)	325	—	Male; infantile genitalia

Following surgery the urinary excretion of iodine was 82 per cent. In April 1951 the basal metabolic rates were −21 and −32 per cent. At that time she appeared myxedematous. Her family had noted that she was less nervous than before the thyroid gland was removed. The prescribed treatment in this case was desiccated thyroid substance and vitamin B complex.

Comment: The rapid pulse and elevated basal metabolic rates gave preoperative evidence of goitrous hyperthyroidism superimposed on longstanding cretinism. Eighteen months following thyroidectomy the patient appeared myxedematous and the basal metabolic rates of −21 and −32 per cent were again considered indicative of the degree of thyroid function. It may be said that thyroidectomy in this instance relieved the patient of pressure symptoms and improved her from the cosmetic standpoint. However, it necessitated continuous thyroid therapy. This last point should be given careful consideration in patients and families of low intellectual level.

CASE II

Gladys C., 29 years of age, was said to have been normal until she was 10 years old when, for the first time, a swelling in her neck was noted. From that time on, her growth rate decreased and her voice became hoarse and she was difficult to understand. She attended the first four grades of school. Although her mother referred to her as being "backward," she admitted that the girl was able to do many things about the house that her older sister (Case I) was unable to do. During the earlier years of her life desiccated thyroid was prescribed but was never taken with consistency. Except for the fact that she menstruated, no accurate menstrual history could be obtained.

Her weight was 97 pounds, height 52½ inches, and pulse rate 98. The measurements

were those of a juvenile. The facies was cretinoid and the characteristics of cretinism prevailed as in Case I. There was a large nodular goiter about the size of a grapefruit. A loud systolic murmur was heard over the thyroid vessels. There was a grade II systolic murmur over the pulmonary area of the heart. There were no other findings of significance.

Laboratory studies: Blood counts, urine, blood sugar, nonprotein nitrogen and serum calcium levels and the results of liver function tests were all normal. The concentration of serum cholesterol was 178 mg. per 100 cc. The glucose tolerance curve was flat. Basal metabolic rate determinations averaged +35 per cent. Following a tracer dose of radioactive iodine there was a urinary excretion of 16.5 per cent. The electrocardiogram was normal. X-ray examination of the chest showed the cardiac shadow to be increased 27 per cent above normal. Study of the long bones revealed flattening of the heads of the femora with shortening of the necks. Epiphyseal centers were fused.

Fluoroscopic examination: There was displacement of the esophagus and the trachea to the left. Calcifications were noted within the area of the thyroid gland. An x-ray film of the skull showed a proportionate increase in the antero-posterior dimensions of the skull; the sella turcica was considered normal and there was a moderate amount of hyperostosis frontalis interna.

Progress: Subtotal thyroidectomy was performed in January 1950. Microscopic examination of removed tissue (Fig. 3) revealed a rather diffuse metaplasia of the acinar epithelium with hemorrhage into the lumens and areas of necrosis. The cells showed a variation in size and shape with hyperchromatic nuclei, multinucleated cells and solid irregular cords of cells. There was no invasion. Colloid was seen within the lumens.

Three months later the basal metabolic rate was −11 per cent. There was no remarkable change in the patient, either from our observation or from that of her mother.

Comment: This girl followed the same pattern as that of her older sister in early life. The family failed to see that she obtained sufficient thyroid medication to avoid a recurrence of her older sister's plight. The thyroid gland was considered to be overactive prior to operation. The pathologic picture was similar to that in the case of her sister. Three months following thyroidectomy there was a lowering of the basal rate from the preoperative level of +35 per cent to −11 per cent. There was no detectable change, however, in the characteristics of the patient.

CASE III

Lorene C., 27 years of age, attended school to the sixth grade. Following that she worked as a domestic for about ten years. For the five years previous to our observation she had been at home because she was too weak and run-down to do any work. She began to menstruate at the age of 16 and had regular monthly periods of five to six days' duration until her second thyroid operation. At the age of 17 she underwent a thyroid operation at Barnes Hospital, St. Louis, and four years later a second thyroid operation was performed. Following this second operation her menstrual interval was from one to three months.

Her weight was 79 pounds, height 58 inches, and pulse rate 80. Perception and response were extremely slow, although answers were fairly intelligent if she was given ample time. The hair was coarse and the skin was dry. Teeth were in fair condition and the tongue was not unusual. She was poorly nourished but showed no other remarkable findings.

Laboratory studies: Blood counts and urine were normal. The basal metabolic rate was +3 per cent (one determination). The serum cholesterol level was 443 mg. per 100 cc. The patient did not return for further studies.

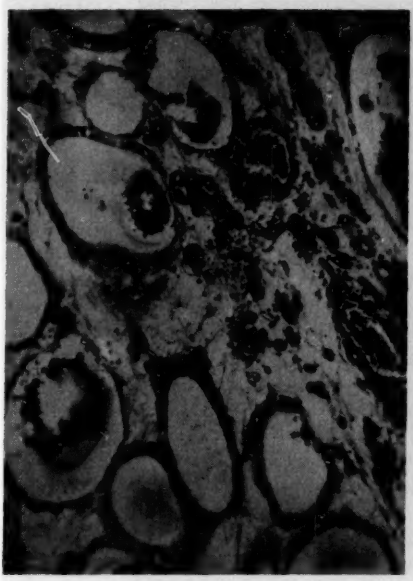

FIG. 2. Section of thyroid gland removed in Case I, showing multiple colloid adenomata with interstitial fibrosis. (See text for description.)

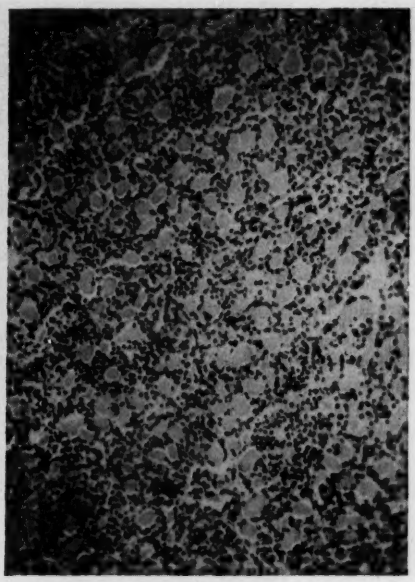

FIG. 3. Section of thyroid gland removed in Case II, showing multiple colloid adenomata with hyperplasia. (See text for description.)

Comment: This girl did not have the classic cretinoid facies but possessed many of the other characteristics of this entity. She had a goiter that was first noticed at the age of 17; following removal, it recurred and required a second operation four years later. During her formative years she failed to grow, as indicated by her height of 58 inches. Even so, her physical and mental development permitted her to carry on as a domestic. The serum cholesterol level of 443 mg. per 100 cc. was considered a better index of thyroid activity than the basal metabolic rate of +3 per cent (single test).

CASE IV

Margaret C., 22 years of age, was started on thyroid medication when she was 3 years of age. It was stated that she seemed to improve on this treatment but became nervous; for this reason her father had refused to take her back to the doctor, even though she failed to grow and develop mentally. She spent little time in school, probably not more than one year, because she could not learn. She began to menstruate at the age of 17 at intervals of two to three months with a duration of four to five days.

Her weight was 94½ pounds, height 51 inches and pulse rate 100. She had cretinoid characteristics with coarse dry skin, thickened tongue and irregular dentition. No goiter was present.

Laboratory studies: Blood counts and urine were normal. The serum cholesterol level was 390 mg. per 100 cc. The basal metabolic rate was −5 per cent. Following a tracer dosage of radioactive iodine the urinary excretion was 58 per cent. An electroencephalogram showed cortical excitability. This patient did not return for further study.

Comment: Thyroid was started at the age of 3, which was too late. Even had it been continuous and in adequate amounts, it could not have been expected to overcome the stigmata of cretinism. The serum cholesterol level of 390 mg. per 100 cc. was to be expected. The 58 per cent urinary excretion of radioiodine following a tracer dose indicated that the gland was able to take up iodine. It lacked, however, the ability to utilize it in the production of the thyroid hormone.

CASE V

Merrill C., 21 years of age, was first noted to be deficient at the age of 1 year. His retardation was erroneously attributed to an attack of whooping cough at the time. He went to school off and on for four or five years. He was able to carry on a few chores around the home. At about the age of 19 he was given thyroid medication for about a year, during which time he grew an estimated 3 inches. The family noted that he did better while on thyroid medication, but for several months before the patient was seen by us they had discontinued it.

His weight was 106 pounds, height 53½ inches, and pulse rate 88. His characteristics were cretinoid, but there was no no goiter. Genitalia showed juvenile development with fine pubic hair. There was sparse axillary hair. He had the general appearance of a child of about 9 years of age. He was unable to answer questions and occasionally uttered a few unintelligible guttural sounds.

Laboratory studies: The basal metabolic rate was −9 per cent. The serum cholesterol level was 325 mg. per 100 cc. X-ray examination of the long bones showed nonunion of the epiphyses. The patient did not return for further studies.

Comment: Again, because of lack of thyroid therapy, this boy's plight was similar to that of four other children in the family. It was of particular interest to note that he was reputed to have gained 3 inches over a period of about one year, at the age of 19, while taking thyroid. There was lack of primary genital development, and secondary sex characteristics were deficient (scant pubic and axillary hair). X-ray examination of the osseous system showed development corresponding to that normally seen in a boy of about 12 years of age (Fig. 4). With the epiphyseal centers ununited, thyroid therapy would be expected to give this boy additional statural growth and improved physical development. The elevated serum cholesterol level was a significant laboratory finding.

CASE VI

Another child of the family consulted us because of goiter. She was not cretinous, but was hyperthyroid with basal metabolic rate of +26 per cent. After preparation with propylthiouracil and iodine, subtotal thyroidectomy was performed. Microscopic section of the thyroid (Fig. 5) revealed acini of varying sizes distended with colloid and lined by flattened or low cuboidal epithelium. There was evidence of both hyperplasia and involution. The tissue was divided into lobules by fibrous septae.

DISCUSSION

The cause of cretinism in a nongoitrous area in a family without a previous history of goiter and thyroid disease is difficult to explain, and all the more so when 5 of 10 siblings were affected. The mother and father were second cousins. Stanbury and Hedge (1) reported the parents of a family of goitrous cretins to be first cousins. Consanguinism has not been recorded

FIG. 4. X-ray film of wrist and hand of M.C., a 21-year-old male. (Case V.) Note the imperfect development of the ossified bone centers and the failure of union of the centers with the epiphyses.

as a possible cause of cretinism in the past and, in these two instances, it may be coincidental. The mother experienced no undue illness during any period of gestation and there was no change in her living habits. Vegetables were eaten freely by the family and it was admitted that a large part of the vegetable diet came from the cabbage family, recognized, experimentally at least, as a source of goitrogens. It is speculated that maternal factors existing during pregnancy accounted for the altered utilization of iodine in the cretin offsprings, rather than a lack of iodine supply. Why goiter developed in 3 of the cretins but not in the other 2 is difficult to explain. From iodine uptake studies[1] made on 3 of the 5 cretins (2 with goiter), there is

[1] At the time this study was made radioautographic technic was not available. This technic, which we now employ, permits a direct study of iodine uptake.

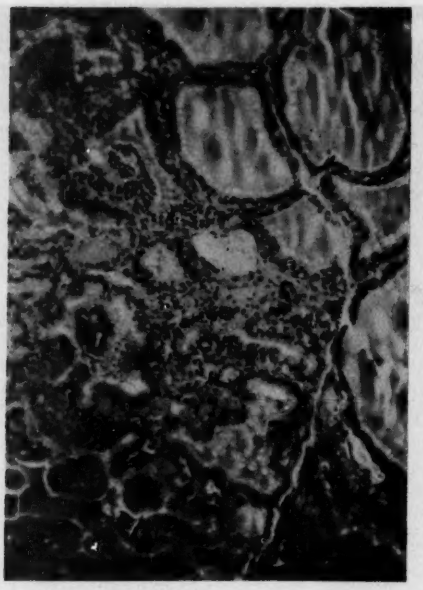

FIG. 5. Section of thyroid gland removed from noncretin hyperthyroid member of family. (See text for description.)

evidence that the gland was able to take up iodine. Stanbury and Hedge (1), in a study of goitrous cretins, advanced the thought that although the enlarged thyroid glands of cretins have the ability to extract iodide from the blood, they do not have the ability to incorporate the iodide into tyrosine. Exceptions to this theory are exemplified by Patients I and II, in whom there was suggestive evidence of hyperthyroidism superimposed on long-standing cretinism—an unusual condition that is difficult to explain. In Patient IV, in whom there was no goiter, the urinary excretion of iodine was 58 per cent, indicating a fair uptake. This observation does not point to thyroid agenesis.

Since cretinism may occur in several members of a family, one should be alert for its occurrence after the first cretin is discovered. It probably is best to institute oral thyroid therapy upon the slightest suspicion of the presence of thyroid deficiency. Probably the best means of detecting it is by observation, being on the alert for characteristics of cretinism such as coarse hair, thick lips, thick tongue, dry skin, heavy features, and inability to feed properly. In the first few months of life there is no single laboratory test that is diagnostic. Serum cholesterol concentration is normally high in infants, creatine-creatinine excretion studies are practically impossible, and

protein-bound iodine studies are difficult. Radioactive iodine studies in nongoitrous cretinism may be of value as an early diagnostic aid, but as yet there is not enough information available to define their value. It has, however, been shown that the goiter of cretinism has a particular avidity for radioactive iodine.

In a suspected case of cretinism, trial thyroid therapy should be employed. In the beginning $\frac{1}{10}$ grain of thyroid in powder form should be given in the milk daily. Rectal temperatures may be used as a guide for dosage. As long as the temperature rise does not exceed 1° F., the dosage should be increased gradually to as much as $1\frac{1}{2}$–2 grains daily. Should the temperature rise above 99.6°F., thyroid should be discontinued. Intercurrent infections will cause increased temperature, but should the rise in temperature be due to thyroid medication it will subside within twenty-four hours after withdrawal of therapy. Once the need for substitution treatment is established, thyroid therapy should be continued indefinitely without interruption, except when necessitated by intercurrent infections or illnesses. Dosage should be maintained at the maximum tolerance level at all times. It is too late to begin treatment at about the ninth month of postnatal life when physical signs of cretinism become evident. If the patient is to approach normalcy, treatment must be instituted by the third month or even earlier.

It has been our privilege to observe 2 cretins at the ages of 15 and 18, respectively, who were given thyroid medication continuously since the age of nine months. Although they had made good progress, both of these children had distinct stigmata of their deficiency.

Less severe forms of congenital and early acquired thyroid deficiency can be successfully treated by early recognition and adequate therapy. In all instances of backwardness in infancy or childhood the possibility of thyroid deficiency should be explored, because it is the one cause of mental retardation for which satisfactory treatment is available.

The retarded osseous development found in hypothyroid states is well demonstrated in Figure 1. A study of the osseous system in suspected hypothyroidism in children is of great diagnostic value.

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Editorial

PITUITARY ADENOMAS

PITUITARY adenomas are of special interest and importance to the internist because of the variety of symptoms encountered. They are nearly all benign tumors of the anterior lobe, and are composed of chromophobic, eosinophilic or basophilic cells. The symptoms that they produce fall into two categories: 1) those due to local pressure, and 2) those due to endocrine dysfunction.

The local pressure symptoms most commonly observed are visual disturbances and headaches. The visual defects, due to pressure on the optic tracts, range from the classic bitemporal hemianopsia to field defects for red and blue without defects for small white objects (1). The headaches do not conform to any single pattern, but are frequently bitemporal. The attention of the physician is soon focussed on the region of the pituitary when local pressure symptoms are present, and an x-ray examination of the sella turcica will usually show abnormalities. Chromophobic and eosinophilic adenomas often cause these symptoms, but basophilic adenomas never become large enough to cause such pressure effects.

Symptoms of endocrine dysfunction result from abnormal secretion of the various pituitary hormones; there may be either increased or decreased secretion of one or more of these hormones in the individual patient. Thus a great variety of clinical pictures may be seen.

Chromophobic adenomas which secrete no hormones comprise at least 70 per cent of the pituitary adenomas which produce symptoms. They may produce no visual disturbances or headaches and yet cause decreased production of pituitary tropic hormones by pressure on the adjacent normal pituitary enclosed in the sella turcica. Recent cytologic study of the anterior pituitary (2) has shown that cells secreting gonadotropins are localized to the upper and lower surfaces of the gland. This explains why chromophobic adenomas invariably depress first the secretion of gonadotropic hormones, if they interfere with anterior pituitary function at all. In premenopausal women, amenorrhea ensues; in males, there is loss of libido and potentia with decrease in testicular size. In older patients, when gonadal function has decreased, gonadotropin deficiency will not be apparent and the first signs will be those of decreased thyroid or adrenocortical function. Since such endocrine symptoms are commonly the presenting symptoms of chromophobic adenomas, it is important for the physician to obtain an x-

ray examination of the sella turcica in any patient with unexplained hypogonadism. In older patients, it is equally important to perform such a study if unexplained hypothyroidism or adrenal insufficiency exists (3).

Chromophobic adenomas characteristically cause a uniform enlargement of the sella turcica, giving it a ballooned appearance. Calcification rarely occurs in these tumors. If calcification is found in an enlarged sella turcica, the lesion is probably a craniopharyngioma. It is not uncommon to find an enlarged sella turcica (greater than 130 sq. mm. in lateral plane area) when a skull x-ray film has been made with some other purpose in mind. These are presumably chromophobic adenomas. Such lesions should not be treated if there are no symptoms, but should be carefully followed. If further enlargement of the sella occurs after three to six months, it is advisable to treat the patient, even if no symptoms have appeared.

Eosinophilic adenomas, comprising about 25 per cent of pituitary adenomas producing symptoms, secrete growth hormone and probably other pituitary tropic hormones. Gigantism results if the tumor occurs before the epiphyses have closed; and acromegaly, if the tumor occurs after they have closed. The sella turcica is enlarged, ballooned or eroded in at least 80 per cent of these patients. As the adenomas increase in size, pressure on the adjacent anterior pituitary frequently results in decreased production of other tropic hormones.

Eosinophilic adenomas often become inactive spontaneously and remain so for years. Before treating patients with these tumors, it is important to determine whether or not the disease is active. This can usually be readily ascertained on the basis of the history and physical findings. When the decision is difficult to make, determination of the serum phosphorus level may prove helpful. It has been found that the concentration of serum phosphorus is usually elevated when acromegaly is active (4). Occasionally, diabetes may be the only evidence of activity in acromegaly; if adequate treatment is given early, the development of permanent (metahypophyseal) diabetes can be prevented.

Basophilic adenomas are found in about 3 per cent of routine autopsies but rarely produce symptoms. These tumors never become large enough to produce local pressure effects or enlargment of the sella, so clinical diagnosis is difficult, if not impossible. Although the original description of Cushing's disease incriminated the basophilic adenoma, it is now generally agreed that the varied clinical manifestations are due to hyperfunction of the adrenal cortex. Although the vacuolizations of the basophilic cells first described by Crooke are commonly found in Cushing's disease, basophilic adenomas are uncommon and are seldom diagnosed before postmortem examination.

Pituitary adenomas may be treated by irradiation or surgery or by a

combination of the two. Roentgen therapy is now considered the initial treatment of choice and surgical intervention is reserved for those patients who fail to respond to adequate irradiation. Exceptions to this general statement, and therefore indications for surgery, are increased intracranial pressure, imminent threat of complete loss of vision, and signs of recent sudden changes such as hemorrhage in the tumor.

Surgical removal of these tumors is technically difficult and the mortality rate is still high—from 5 to 10 per cent. Recurrences are common despite postoperative irradiation, which should be begun within the month after surgery. Marked hyperthermia commonly occurs during the immediate postoperative period and hypopituitarism often follows operation because of damage to the adjacent normal pituitary.

Roentgen therapy methods have improved to such a degree that successful results can be achieved in about 70 per cent of pituitary adenomas. It was formerly feared that radiation therapy would aggravate the local pressure effects, but increasing experience with judicious application has largely allayed this fear. Aggravation of signs and symptoms seldom occurs, and promptly subsides when treatment is interrupted. Treatment failures are thought in many instances to be due to cystic changes in the tumor, the existence of which it is impossible to predict.

Although not highly radiosensitive, pituitary adenomas of all types are more sensitive to roentgen rays than are the normal pituitary and the tissues surrounding it. In the past, results of roentgen treatment were discouraging because the dosages employed were too small. It was feared that larger doses might damage soft tissue, bone or brain. In the last five years Kerr (5), Bachman and Harris (6), and Buschke (7) have used much larger dosages with no ill effects and with increased success. It has been found by all these therapists that larger dosages administered continuously over a short period of time, through multiple portals, are far superior to small dosages in repeated short courses at intervals of a few weeks.

Johnsen (8) has recently reported uniformly favorable results in treating acromegalics with large roentgen dosages. Very intensive short courses of 3,000 r measured in air were given at intervals of three to six months until the disease was arrested. The author considers persistent increase in the soft tissues as evidence of activity of the disease and recommends that all acromegalics with such changes receive a total of 9,000 r measured in air. This is presumably equivalent to a tumor dose of about 3,000 r. In his 23 cases, no harmful effects were noted.

One can conclude, therefore, that roentgen therapy of pituitary adenomas is effective in most cases and is quite safe. The visual fields should be checked before treatment is started. If there are defects, the fields should

be checked at weekly intervals during treatment. Minor decreases in the visual fields, unassociated with symptoms, do not warrant interruption of therapy. Epilation uniformly occurs but is nearly always temporary. Nausea and vomiting occasionally occur early in treatment and respond satisfactorily to pyridoxine, 50 mg. three times a day by mouth. If signs of increased intracranial pressure appear, neurosurgical advice should be obtained immediately.

The tumor dose should be at least 3,000 r and should be given in a single course over a period of four or five weeks. Treatment is given five or six days a week during this period, through three or four 5 to 6 cm. portals. The use of full beam direction technique is essential if the tumor dose is to be delivered accurately. Doses expressed as r measured in air or on the skin are unreliable because of the variable relationship between such figures and the actual tumor dose. This relationship depends not only on the size and shape of the patient's head, but on the quality of beam employed, the size and arrangement of the skin fields, and the distance separating the skin from tube target. In each patient, the tumor dose will be equivalent to a different total skin dose because of these many variables.

It is wise to start with small daily increments, on the order of 100 r to the skin of a single field. The daily dose is increased as rapidly as seems safe, to the daily requirement that has been calculated as necessary to complete the treatment in four or five weeks. This requirement will usually be 200 to 300 r, to the skin of two of the fields at each sitting. If three or four portals are employed, the total dose to each will fall well within safe tolerance limits.

Improvement may begin during the course of treatment but often does not become apparent for months. If the patient fails to improve, or has a relapse after improvement has occurred, it is probably safe to repeat the same course of treatment after an interval of six months. It should be realized, however, that improvement is less likely to follow a second course of such treatment than the first. If the patient's condition becomes worse, surgical intervention should be recommended.

Hormone treatment of eosinophilic adenomas has been attempted by a few investigators. Kinsell *et al.* (9) have shown that both estrogens and androgens produce a fall in the serum phosphorus level. It is interesting that testosterone propionate but not methyltestosterone produced this effect. Although some temporary benefit has apparently been achieved by the use of hormones, the effect lasts only as long as they are administered.

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Questions and Answers

Answer by R. M. Archibald, Hospital of the Rockefeller Institute, New York, N. Y.

QUESTION: Does ACTH act at the cell membrane or intracellularly? At what concentration does it become effective in stimulating release of hormone?

ANSWER: If by this question is meant, "At what concentration at the cell membranes or in the cells of the adrenal cortex will ACTH stimulate release of hormone?", I doubt if anyone knows the answer. Sydnor and Sayers, *J. Clin. Endocrinol. & Metab.* **12**: 920, 1952, have indicated that normal human adult male blood contains less than 1 milliunit per 100 ml., whereas the level in patients with Addison's disease is 2 to 4 milliunits per 100 ml. Presumably, the level in blood of normal individuals is high enough to permit normal functioning of the cells of the adrenal cortex, but this procedure requires 18 ml of blood for each animal used in the assay. The concentration of ACTH in intracellular fluid may be different. More sensitive techniques for assay will be required before this question can be answered in a satisfactory manner. Prerequisite to any statement regarding concentration of ACTH in cell membranes would be not only a sensitive assay method, but also either the selection of an arbitrary thickness of the membrane and measurement of areas of cells, or an expression of concentration in terms of cell area with the assumption that one was concerned with a monomolecular layer.

If the question is intended to ask, "What is the mechanism by which ACTH stimulates production or release of hormone by the cells of the adrenal cortex?", it is again necessary to profess ignorance. This is the same question which during the past several years an increasing number of scientists have been asking themselves. So far there is no satisfactory clue as to the mechanism by which hormones exert their influence. This point has been discussed by Pincus in his Presidential Address, *J. Clin. Endocrinol. & Metab.* **12**: 1190, 1952.

Answers by Norris J. Heckel, Chicago, Ill.

QUESTION: What is the effect of cortisone on cancer of the prostate?

ANSWER: Huggins has demonstrated that certain prostatic cancers are dependent upon the function of the adrenal gland and some patients have an amelioration of the disease after bilateral adrenalectomy. He and others have observed that some patients with advanced prostatic cancer who have a relapse after anti-androgenic therapy may be benefited by the administration of 5 mg. of stilbestrol and 50 mg. of cortisone a day.

QUESTION: Is it practical to use estrogen therapy after castration? If so, in what amounts?

ANSWER: Yes, it is not only practical but efficacious. In order to evaluate the therapeutic results of orchiectomy and estrogen therapy in prostatic cancer, Nesbit and Baum collected data on 1,818 patients. It was their conclusion that the 5-year control of prostatic cancer is more effectively obtained by combined treatment, *i.e.*, orchiectomy and stilbestrol, in patients who are free of metastases. The usual dose of stilbestrol is 1 mg. two or three times a day.

QUESTION: In the treatment of prostatic cancer, should estrogens be used first and orchiectomy be reserved until further symptoms occur, since there may be further benefit following this procedure whereas none occurs if the procedures are reversed?

ANSWER: This is not necessarily true. According to Nesbit and Baum, orchiectomy is more efficacious than estrogens when metastases are present. In the management of patients who have a relapse there is no advantage of one form of therapy over the other.

QUESTION: Does TACE have any advantage over stilbestrol in the treatment of prostatic cancer?

ANSWER: It is thought by some that TACE will not produce as much soreness and enlargement of the breasts. Otherwise, in our experience, it has no advantage over stilbestrol.

QUESTION: What treatment do you recommend for unilateral undescended testis?

ANSWER: The type of treatment depends upon the age of the patient. For instance, after the age of puberty the treatment is orchiopexy if there is no evidence of an endocrine disorder such as eunuchoidism or Fröhlich's syndrome. When either one of these conditions is present then androgens or one of the gonadotropins should first be given. If the testis has not descended by the time the desired genital growth has occurred, orchiopexy is necessary.

Before the age of puberty one of the gonadotropins should first be given—preferably 500 I.U. three times a week and usually for a period of six to eight weeks. If the testis has not descended by the end of this period the chances are that this form of therapy will not succeed and an orchiopexy is necessary.

Answers by E. Perry McCullagh, Cleveland Clinic, Cleveland, Ohio

QUESTION: What is the derivation of the term "euthyroidism?"

ANSWER: The term "euthyroid" is derived from the Greek prefix "eu" meaning good, advantageous, well, easily, and is the opposite of "dys." Euthyroidism is used in modern Greek medicine and means exactly what it means in English, namely a state of normal thyroid function.

QUESTION: Is there any difference in sodium and water retention during methyltestosterone therapy, as compared with testosterone propionate therapy?

ANSWER: Although much work has been done with regard to the sodium and water retaining properties of androgens, very little has been published on a direct comparison between this property in methyltestosterone as compared with testosterone propionate. Quotations which have a bearing are as follows: "In the latter case (case of eunuchoidism reported by Bassett, 1945) there was an apparent difference between the action of methyltestosterone and testosterone propionate. The retention of chloride after administering the first compound was delayed and, on cessation of treatment, chloride was excreted immediately. Testosterone propionate, on the other hand, caused an immediate retention of the chloride followed by a gradual and extended loss on cessation of injections." (Kochakian, C. D.: The protein anabolic effects of steroid hormones, *in* Vitamins & Hormones **4**: 255–310, 1946.)

The Endocrine Society

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTY-SIXTH MEETING

THURSDAY, FRIDAY AND SATURDAY, JUNE 17, 18 AND 19, 1954

Sir Francis Drake Hotel

San Francisco, California

(Abstracts arranged according to numbers on the printed program)

THURSDAY, JUNE 17, 1954

9:30 A.M. GONADS, GONADAL HORMONES AND GONADOTROPINS

CHAIRMEN: WARREN O. NELSON AND THOMAS H. MCGAVACK

1. Action of Pregnanediols and Related Steroids on Progesterone in Decidual Development.

Frederick L. Hisaw, Ph.D., Joseph T. Velardo, Ph.D. (by invitation) and *Harry K. Ziel, A.B.* (by invitation).

The Biological Laboratories, Harvard University, Cambridge 38, Mass.

It has been reported that pregnanediol will inhibit the action of progesterone in the development of deciduomata in rats (Hisaw and Velardo, 1951). Inhibition was determined on pseudopregnant rats that were ovariectomized on the fifth day, one uterine horn traumatized, 1.5 mg. of progesterone and the compound being tested injected at separate sites daily, and the uterus examined at 72 hours. It has since been found that impure preparations of pregnanediol from pregnancy urine that are not estrogenic or androgenic vary widely in inhibitory action. A series of purified steroids from pregnancy urine and synthetic compounds has been tested. Pregnanediol (6.0 mg. daily) and pregnanediol diacetate (5.0 mg.) inhibited the response less than 25 per cent, whereas crude pregnanediol ($\frac{1}{4}$ to 12.0 mg.) inhibited it 50 per cent. No pregnanediol glucuronide at the 5.0-mg. level was without effect in inhibiting progesterone. In testing synthetic pregnanediols and other steroids, it was observed that pregnane-3α,20α-diol did not inhibit, pregnane-3α,20β inhibited minimally, and pregnane-3β,20β-diol inhibited progesterone 75 per cent. Pregnanedione at 1 milligram inhibited progesterone 75 per cent, allopregnanedione did not inhibit, both pregnane-3-ol,20-one and allopregnane-3-ol,20-one acetate inhibited 25 per cent at 5.0 mg. daily, and 17-OH-progesterone inhibited 35 per cent at 3.0 mg. daily. Variations in inhibitory action of urinary preparations is probably due to the difference in the steroid compounds contained in urine.

2. Control of Menopausal Vasomotor Symptoms with Pituitary Luteinizing Factor and Chorionic Gonadotropin.

Arthur A. Hellbaum, Ph.D., M.D., James B. Eskridge III, M.D. and *Richard W. Payne, M.D.*

Dept. of Pharmacology and Obstetrics, University of Oklahoma School of Medicine, Oklahoma City, Okla.

We have previously shown that estrogens stimulate the release of the pituitary luteinizing (LH) factor while inhibiting the follicle-stimulating (FSH) factor in menopausal women. The concentration of LH is relatively low in the blood and urine of patients with menopausal vasomotor symptoms, whereas the FSH concentration is high. In the present study, 36 oophorectomized women with severe vasomotor symptoms were followed after the administration of various gonadotropic substrances. The vasomotor symptoms were alleviated by those preparations containing the LH factor by itself, and unfractionated preparations containing both FSH and LH. The response to chorionic gonadotropin was similar to that of LH. FSH by itself was without effect on menopausal women.

3. Effects of Estradiol on the Metabolism of Human Placenta.

Claude A. Villee, Ph.D. (introduced by Paul L. Munson).

Depts. of Biological Chemistry and Obstetrics, Harvard Medical School, Boston, Mass.

Homogenates obtained from term placentas were prepared in 0.25 M sucrose and incubated in a phosphate-buffered medium containing 1 mM of citrate, 40 mM of K^+ and 10 mM of Mg^{++} per liter. The medium was fortified with cytochrome c, ATP and DPN. Estradiol at a level of 1 microgram per milliliter increased oxygen consumption 47 per cent, citrate utilization 57 per cent and ketoglutarate production 47 per cent over the control values. The effect of estradiol on citrate utilization was evident after a 15-minute incubation, and that on ketoglutarate production after a 30-minute incubation. A soluble enzyme system, obtained as the supernatant fluid when the homogenate was centrifuged sixty minutes at 57,000×gravity, did not utilize oxygen but did utilize citrate and accumulate ketoglutarate. This enzyme system was stimulated more than 100 per cent by the addition of estradiol. The citrate of ketoglutarate reaction will occur anaerobically in the presence of DPN; under these conditions there is an accumulation of lactate. The estradiol stimulation is evident anaerobically as well as aerobically. These effects were evident in preparations from placenta or endometrium but not in those from liver or kidney. The data suggest that estradiol stimulates one or more of the enzymes in the citric acid cycle and not some enzyme in the coupled electron transmitter system.

4. Metabolism of the Natural Estrogens in Men with Laennec's Cirrhosis of the Liver.

Benjamin F. Stimmel, Ph.D.

Rees-Stealy Clinic Research Foundation, San Diego, Calif.

The exogenous urinary estrogen excretion patterns (estradiol-17β, estrone, estriol) of 6 men hospitalized with terminal (Laennec's) cirrhosis of the liver were determined spectrophotometrically, after treatment with a single, small (5.0 mg.) dose of one of the natural estrogens. The following observations were made: 1) The metabolic patterns—estradiol-17β > <estrone >estriol conversion and conjugation—are qualitatively the same in the cirrhotic patients as in healthy men. 2) Of the 6 patients, 2 who had the most serious impairment of liver function as shown by the degree of urobilinogenuria demonstrated virtually no capacity for conjugating excreted estrogens. 3) All 6 patients converted estradiol-17β to estrone and estriol, but the estrone/estradiol-17β and estriol/estrone ratios were lower than those in healthy men. 4) Total estrogen recovery values were

elevated in the 6 cirrhotic patients, but these values accounted for less than 20 per cent of the administered estrogen. Paradoxically, all 6 patients, when treated with estriol (free), were capable of excreting it in conjugated form.

5. Amenorrhea with Normal Follicle-Stimulating Hormone (FSH): Follow-Up Report of 60 Cases.

Patricia H. Benedict, M.D. and *Fuller Albright, M.D.*

The Ovarian Dysfunction Clinic and the Dept. of Medicine, Massachusetts General Hospital, and the Dept. of Medicine, Harvard Medical School, Boston, Mass.

With respect to the level of their FSH excretion in the urine, patients with amenorrhea may be divided into three groups: (a) with low FSH excretion, when the initial disorder is presumably in the pituitary; (b) with high FSH excretion, when the initial disorder is in the ovary; and (c) with FSH excretion neither low nor high (so called "hypothalamic" amenorrhea). It is with the last of these three groups that the present paper is concerned. Just what constitutes the primary disorder in this group remains obscure; there is a question of hypothalamic disorder leading to the absence of luteinizing hormone, or a question of some disorder of estrogen or other steroid metabolism. It would seem that one must also hypothesize the presence of an FSH-inhibiting, nonestrogenic steroid. Regardless of the hormonal pattern, these cases present a definite clinical picture which must be thought of as a clinical entity. Analyzed in this study are ages of patients when seen, duration of amenorrhea, and incidence of other endocrine disease. Emphasis is placed on whether or not menstruation occurred following a course of progesterone, used as a diagnostic procedure. The incidence of return of menses is presented.

6. Suppression of Ovulation in the Rat by Morphine.

Charles A. Barraclough, Ph.D. (by invitation) and *Charles H. Sawyer, Ph.D.*

Dept. of Anatomy, University of California at Los Angeles School of Medicine, and Section of Investigative Medicine, Veterans Administration Hospital, Long Beach, Calif.

Morphine addiction causes amenorrhea and sterility in the human female, but the mechanism of this action is unknown. The spontaneity of the human ovarian cycle has an analogue in that of the rat. The finding, by Everett *et al.* (*Endocrinology* **44:** 234, 1949; **47:** 198, 1950) that "spontaneous" ovulation in the rat involves a neurogenic timing factor with a 24-hour rhythmicity provides an experimental basis for investigating whether morphine induces sterility by blocking neurogenic activation of ovulation. Normally, the neurogenic stimulation involved in the release of pituitary ovulating hormone occurs between 2:00 and 4:00 P.M. of the day of proestrus. Injection of 5-day cyclic rats with morphine sulfate (50 mg./Kg., S.C.) between 12:00 and 2:00 P.M. of proestrus resulted in failure of ovulation in 13 of 16 animals. In contrast, treatment at 4:00 P.M. was followed by ovulation in 10 of 10 rats. Results with animals examined forty-eight hours after a single injection at noon of proestrus confirmed the 24-hour rhythmicity: 4 of 4 had ovulated the second night. Injection of morphine daily at noon for periods up to eight weeks apparently suppressed ovulation for the duration of treatment in 2 rats. These results suggest that morphine induces sterility by blocking neurogenic stimulation of the release of pituitary ovulating hormone.

7. A Sensitive Antihormone Assay Method Employing Chorionic Gonadotropin as the "Test Hormone."

William O. Maddock, M.D., Robert B. Leach, M.D. (by invitation), *Ichiro Tokuyama, M.D.* (by invitation) and *C. Alvin Paulsen, M.D.*

Dept. of Medicine, Wayne University College of Medicine, Detroit, Mich.

Some pituitary gonadotropin must be present for chorionic gonadotropin (C.G.) to stimulate function of the immature rat ovary. This suggested a sensitive method for detecting antihormones; to prevent the ovarian stimulating action of C.G., antihormones need block only the presumably minute amounts of endogenous gonadotropin circulating in the immature female rat. When C.G. was substituted for hog FSH as the "test hormone" in assaying the plasma antihormone levels of 6 patients who received hog FSH for two to three months, only 1/10 to 1/20 the amount of plasma was needed to detect antihormone activity. Tested against C.G., antihormones still could be detected twelve months after FSH treatment whereas, using FSH, they could be detected for only two months. The following observations support the interpretation that the antihormones do not actually block C.G., but act by neutralizing the assay rats' endogenous gonadotropins. When tested directly, the antihormones did prevent the action of rat pituitary gonadotropin, but in immature *male* rats, did not inhibit the direct, Leydig cell-stimulating action of C.G., which does not require the presence of pituitary gonadotropin. Two men having high antihormone levels as a result of FSH therapy responded promptly to C.G. therapy as judged by symptoms and by increased urinary estrogens and 17-ketosteroids.

8. Hyperadrenocorticism and the Stein-Leventhal Syndrome.

Najib Abu-Haydar, M.D. (by invitation), *John C. Laidlaw, M.D.* (by invitation), *Bernardo Nusimovich, M.D.* (by invitation), and *Somers Sturgis, M.D.* (by invitation) (introduced by George W. Thorn, M.D.)

The Depts. of Medicine and Surgery, Harvard Medical School, and the Medical and Surgical Service, Peter Bent Brigham Hospital, Boston, Mass.

The polycystic ovary syndrome, as described by Stein and Leventhal, is characterized clinically by amenorrhea and sterility, frequently hirsutism and occasionally obesity. Pathologically, there is enlargement of the ovaries with a thick tunica albuginea, many follicular cysts, and hyperplasia of the theca interna. Some measure of improvement has been obtained in these patients by wedge resection of the ovaries. Bergstrand (*Virchows Arch. f. path. Anat.* **293**: 413–428, 1934) described similar ovarian changes in a virilized patient with a pituitary basophilic adenoma and adrenocortical hyperplasia. The authors have observed ovarian lesions similar to those reported by Stein and Leventhal in a virilized patient with adrenocortical carcinoma and in a woman with acromegaly, masculinization and increased urinary 17-ketosteroids. Furthermore, in a patient who showed the clinical and pathologic picture of the Stein-Leventhal syndrome, there was a high urinary level of 17-ketosteroids; following cortisone administration, the 17-ketosteroid excretion fell to low values. It would thus appear that certain cases of the Stein-Leventhal syndrome may be associated with adrenal hyperfunction. A study of the hormonal mechanisms involved in this syndrome is presented.

9. Chromatographic Patterns of the 17-Ketosteroid Excretion in Men with Reduced Testicular Activity.

Sidney Pearson, Ph.D., Thomas H. McGavack, M.D. and *Helmut Haar, M.D.*

The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

In 2 hypogonadal and 1 agonadal male whose ages were 63, 67 and 43, respectively, the total urinary 17-ketosteroid excretion and chromatographic patterns were detailed after the separation of the ketosteroids into 44 fractions. Fifty per cent or more of the total 17-ketosteroid excretion was found in fractions 33 through 44, which include etiocholanolone, 11-oxysteroids and some, thus far, unidentified steroidal material. Compared with normal "young" individuals, there was a loss of steroidal material in the regions where epiandrosterone, dihydroepiandrosterone and androsterone usually appear. The chromatograms of these patients are compared with those of normal "young" and aged males. The influence of anabolic steroids on such patterns is indicated.

10. The Role of Various Tissues in the Metabolism of Androgens.

Charles D. Kochakian, Ph.D., Donald I. Hamm, M.S. (by invitation), *Dan M. Nall, M.S.* (by invitation) and *George H. Stidworthy, M.S.* (by invitation).

Oklahoma Medical Research Foundation, Oklahoma City, Okla.

Previous studies have indicated that slices of liver and kidney from the rabbit intraconvert testosterone, androstene-3,17-dione and epitestosterone. Present studies indicate that androstane-3,17-dione and androsterone also are formed from androstene-3,17-dione by homogenates of rabbit liver and kidney. Furthermore, androstanedione in trace amounts is formed by skeletal and heart muscles. On the other hand, the liver and kidney of the guinea pig do not contain the enzyme which is responsible for the formation of epitestosterone but possess an enzyme capable of producing epiandrosterone from androstene-3,17-dione but not from testosterone. The addition of testosterone (labeled 4-C^{14}) to a liver homogenate containing androstene-3,17-dione, nicotinamide, and diphosphopyridine-nucleotide results in the inhibition of the formation of androstane-3,17-dione, androsterone and epiandrosterone but the usual conversion of testosterone to androstenedione is observed. When androstanedione is incubated with liver, kidney or heart homogenates, androsterone and epiandrosterone are formed but when skeletal muscle homogenate is used, only epiandrosterone is formed.

Alteration of the pH of the buffer between 6.4 and 8.8 does not affect the nature of the metabolites formed by incubation of liver homogenate with androstene-3,17-dione or androstane-3,17-dione.

These studies indicate the presence of a number of enzymes which are highly specific in their action on the androgens.

11. The Kidney β-Glucuronidase Response to Androgens.

William H. Fishman, Ph.D. (introduced by Dr. F. Homburger, M.D.).

The Cancer Research and Cancer Control Unit, Tufts College Medical School, Boston, Mass.

A system for the assay of steroids for their ability to stimulate renal β-glucuronidase (*Endocrinology* **52**: 536, 692, 1953) has been defined. Seven mg. of each steroid is administered in oil subcutaneously in divided doses once every two days for fourteen days to each of 10 A/Cloudman male mice of approximately 20–25 grams in weight maintained on a synthetic high-protein, high-fat diet. β-Glucuronidase assays of kidney homogenates averaged 3140 ± 420 units/Gm. in control groups; and of urine, approximately 10,000 units/100 ml. of urine. In groups treated with testosterone propionate

these figures were 50,500 ± 2,280 and 1,500,000 (days 7 to 14), respectively. The steroids tested range themselves in order of potency as follows: testosterone propionate, testosterone β-cyclopentylpropionate, methylandrostanediol, methylandrostenediol, androstane-17-β-ol-3-one, methyltestosterone, 19-nortestosterone, testosterone,9,11,-dehydrotestosterone, 11β-hydroxytestosterone, 11α-hydroxytestosterone, vinyl-testosterone, androstanedione-3,17,adrenosterone, androstane-3 β-ol,-17-one, 4-androstene-3,17-dione, epitestosterone and 11β-hydroxy-4-androstene-3,17-dione. Certain inferences may be drawn regarding the relationship of chemical structure of these substances to their renal β-glucuronidase stimulating activity. This biochemical assay correlates better with the myotrophic property of androgens than it does with their virilizing potency.

12. Sexual Differences in Nuclei of Human Skin.

Eve Marberger, M.D. (by invitation) and *Warren O. Nelson, Ph.D.*

University of Iowa College of Medicine, Iowa City, Iowa.

A recent report (Moore *et al.: Surg., Gynec. & Obst.* **96**: 641, 1953) indicates the existence of sexual differences in the nuclear pattern of human cells. In females, skin biopsies showed that 69 per cent of the epidermal nuclei contained a mass of chromatin close to the nuclear membrane. Such masses were seen in but 5 per cent of nuclei in males. To date we have studied skin biopsy specimens from 21 males and 9 females, including cases of hypospadias, cryptorchidism and 3 patients with female pseudohermaphrodism (one having been raised as a boy). The average percentage of nuclei with chromatin masses in females was 64, with a range of 51 to 77 per cent, the pseudohermaphrodites showing 60, 67 and 71 per cent. In males the average percentage was 4, with a range of 2 to 6 per cent, the cases of cryptorchidism and hypospadias falling within this range. Abdominal skin fixed in Davidson's solution and stained with Harris hematoxylin proved in general to provide the most satisfactory preparations, but skin from other areas and prepared in other ways may be used when necessary.

2:00 P.M. THYROID, THYROID HORMONES AND THYROTROPIN

CHAIRMEN: PAUL STARR AND SIDNEY C. WERNER

13. Further Evidence that Graves' Disease Is Hyperthyroidism and Not Hyperpituitarism: Effects of Triiodothyronine and Sodium Iodide.

S. C. Werner, M.D., M. Spooner, M.D. (by invitation) and *H. Hamilton, M.D.* (by invitation).

Dept. of Medicine, Columbia University College of Physicians & Surgeons, and the Presbyterian Hospital, New York, N. Y.

Recent evidence (*J. Clin. Endocrinol. & Metab.* **12**: 1561, 1952) indicates that Graves' disease is a primary disorder of the thyroid and not one mediated through the anterior pituitary. This conclusion is further strengthened by the present studies. Triiodothyronine administered to 10 hyperthyroid patients in a dose of 2 mg. daily (equivalent to about 10 mg. of *l*-thyroxine or 3 Gm. of thyroid daily) caused no significant reduction in 24-hour I^{131} uptake (71 per cent before; 63 per cent after). Sharp decreases followed 0.07 mg. daily in nontoxic diffuse goiter (53 per cent before; 15 per cent after) and in control subjects (28 per cent before; 7 per cent after). Sodium iodide, 11.2 mg. daily, administered to 20 hyperthyroid patients decreased I^{131} uptake sharply (61 per cent before; 16 per cent after) and SPI (11.3 gamma per 100 ml. before; 8.5 gamma per 100 ml. after).

Thyrotropin for three days resulted in little increase in uptake (16 per cent before; 24 per cent after) but a sharp increase in SPI (8.5 gamma per 100 ml. before; 13.0 gamma per 100 ml. after). The improvement caused by iodide therapy, despite responsiveness to exogenous thyrotropin, is inconsistent with an endogenous thyrotropin excess as the cause of Graves' disease. The dissociation between iodine uptake and hormonal output after thyrotropin injection suggests that iodides in Graves' disease act directly upon the thyroid cell and do not inactivate thyrotropin or inhibit the hormone's action at the cell surface.

14. Effects of Iodide on the Rate of Release of Hormone from Toxic Thyroid Glands.

Richard E. Goldsmith, M.D. (introduced by Virginia Esselborn, M.D.)

The effect of iodide ion on the toxic human thyroid gland has been studied for many years. There is evidence showing a decrease in proteolytic enzymes in iodine-treated toxic glands as compared to untreated toxic thyroids. It has also been shown that incubation of TSH with iodide will inactivate the usual stimulating effect of TSH on the iodide uptake of thyroid slices. The present study demonstrates the *in vivo* effect of iodide ion on the rate of release of radioiodine-labelled hormone from the methyl mercaptoimidazole-blocked thyroids of patients with hyperthyroidism. Nine such patients were studied, including controls. The data show that iodide produced a statistically significant slowing of the rate of release of thyroid hormone. When TSH and iodide were administered simultaneously there was a neutralization of the effects on hormone release expected when each is given alone (TSH accelerates and iodide decelerates). The iodide effect is therefore thought to be brought about by interference with TSH activity.

15. Metabolic Effects of Thyrotropic Hormone.

Joseph E. Rall, M.D., Ph.D., Jacob Robbins, M.D. (by invitation), *Chas. D. West, M.D., Ph.D., Olof H. Pearson, M.D.* and *R. W. Rawson, M.D.*

Memorial Center for Cancer and Allied Diseases, New York, N. Y.

Thyrotropic hormone was given to 2 patients in complete metabolic balance.

The first patient was a woman aged 71 with osteoporosis and no demonstrable endocrine disease. A single intramuscular dose of TSH (90 mg. equivalents or 36 I.U.) resulted in the following: a negative nitrogen and phosphorus balance and a creatinuria beginning in twenty-four hours, and lasting nine days. The BMR rose from +2 per cent to +23 per cent in three days while the serum cholesterol level fell from 178 mg. to 150 mg. per 100 ml.

The other patient was a woman aged 41 with primary myxedema. TSH in a dosage of 30 mg. (12 I.U.) given intramuscularly daily for five days had no effect on any of the indices measured.

The metabolic changes after TSH were dependent upon the presence of an intact thyroid and were similar to those produced by both thyroxine and triiodothyronine. The chronologic sequence of changes, however, was most compatible with the hypothesis that both triiodothyronine and thyroxine were released after TSH.

16. An Inquiry into the Levels of Integration of Thyroid Function in Goitrogen Treated Rats.

Nicholas S. Halmi, M.D.

Dept. of Anatomy, State University of Iowa, Iowa City, Iowa.

In addition to its well-known regulation by thyrotropin (TSH), the iodide-concentrat-

ing mechanism (ICM) of the thyroid is subject to influences that do not act through the pituitary, since propylthiouracil (PTU) increases the thyroid serum iodide concentration (T/S) ratio of hypophysectomized rats. By giving graded doses of TSH, with or without concomitant PTU treatment, to hypophysectomized rats and recording follicular cell heights and T/S ratios, it could be ascertained a) that the effectiveness of TSH on thyroid morphology was increased approximately twofold by PTU, without any change in the slope of the response curve, and b) that PTU had a much more striking and not merely quantitative effect on the response of the ICM to TSH. It is suggested that goitrogens a) promote the output of TSH by the pituitary, b) enhance the overall effectiveness of TSH, c) activate the ICM of the thyroid independently of TSH and d) increase the responsiveness of the ICM to TSH.

17. Urinary Excretion of Thyrotropic Hormone by Normal Humans and by Subjects with Altered Thyroid Function.

Joseph P. Kriss, M.D. and *Francis S. Greenspan, M.D.*, with the technical assistance of *William Lew, B.S.* (by invitation).

Dept. of Medicine, Stanford University School of Medicine, San Francisco, California.

An improved method for bioassay of thyrotropin is employed which permits the detection of quantities as low as .0005 U.S.P. unit. The bioassay involves the measurement of radioactive phosphorus (P^{32}) by the chick thyroid six hours after intracardiac injection of the test material. Human urine is processed by ultrafiltration, and the total protein extract is further purified by precipitation with 40 per cent alcohol to eliminate nonspecific protein, and reprecipitated with 80 per cent alcohol. The final dried precipitate from urines of normal subjects when tested by this bioassay procedure is shown to contain about .01–.025 U.S.P. units of thyrotropin per twenty-four hours. Corticotropin, follicle-stimulating hormone, prolactin, and equine anterior pituitary extract do not affect the thyroidal P^{32} uptake. Our experiments indicate, therefore, that a positive response in the bioassay is a specific one due to the presence of thyrotropin. Data are presented showing the variations of thyrotropin excretion in normal subjects as well as amounts excreted by patients with altered thyroid function.

18. A Rapid Simple Clinical Radioactive Method for Evaluating Thyroid Function.

Wm. A. Reilly, M.D. (by invitation) and *K. G. Scott, Ph.D.* (by invitation) (introduced by Peter H. Forsham, M.D.)

Radioisotope Unit, Fort Miley Veterans Admin. Hospital, and the Depts. of Pediatrics and Radiology, Univ. of California School of Medicine, San Francisco 22, Calif.

Patients were given 100 to 150 microcuries of I^{131} (10 to 15 microcuries for children). Plasma (1 to 3 cc.) at intervals up to ninety-six hours was treated with anionic resin (25 per cent suspension). Ninety-five to 98 per cent of the inorganic iodide is adsorbed by the resin, and the organic iodine (hormonal) is left in the supernatant plasma. Scintillation crystal counting is performed on the two fractions. Plasma supernate counts/total counts of plasma are corrected for a uniform dose (100 microcuries), uniform sample (100 cc.) and weight (70 Kg.). This corrected figure, c/s per cent, measures the rate of hormone production.

Data are presented on 47 adults:

	Euthyroid (27 cases)			*Hyperthyroid* (14 cases)		
	3°	24°	72°	3°	24°	72°
c/s %	0	4.11	17.3±1.8	25±11	136±30	198±62

	Hypothyroid (6 cases)		
	24°	48°	72°
c/s %	0	2.9	1.4

For the diagnosis of hyperthyroidism, determinations may be made at 3°; for the diagnosis of hypothyroidism at 48° or 72°.

Data on children (11 euthyroid, 3 hyperthyroid, and 4 hypothyroid) show a higher c/s per cent and indicate that they may form hormone earlier than adults.

Borderline states of function may be fairly well differentiated by this method. This new method (anionic resin+I^{131}) is simpler, easier, and possibly more accurate for measuring hormone production than other methods (PBI, or RBC/PI ratio).

19. The Metabolism of Inorganic Iodide in Thyroid Slices, with Delineation of a New Iodide Compartment.

John L. Bakke, M.D. and *Nancy Lawrence, M.S.* (by invitation).

Veterans Admin. Hospital, and the Dept. of Medicine, University of Washington, Seattle, Wash.

Experiments with beef thyroid slices confirmed the *in vivo* observations of Wolff and Chaikoff that a high plasma inorganic iodide level apparently blocks normal organic binding of iodine.

Because it seemed teleologically doubtful that a high serum iodide level could stop all thyroid hormone synthesis, other interpretations of these observations were explored. Experiments demonstrated that when the inorganic iodide compartment (I.I.C.) was loaded in I^{131} media containing propylthiouracil to block all organic binding and then incubated in either high or low iodide media and unblocked by rinsing away the propylthiouracil, the organic binding of iodide appeared normal, even in high-iodide media.

The apparent conflict between these data may be harmonized by substituting for the usual single iodide pool a model with two hypothetical inorganic iodide compartments ($I.I.C._1$ and $I.I.C._2$) in series anterior to organic binding. These two compartments appear to be delineated by a high iodide level which blocks entry of iodide into $I.I.C._2$, or by KSCN which empties $I.I.C._1$ back into the media leaving $I.I.C._2$ intact. The rate of iodide uptake into $I.I.C._1$ is 1) increased in high-iodide media, 2) increased by adenosine, 3) increased by TSH in doses as small as 0.019 milliunit (U.S.P.), 4) blocked by SCN^-, and 5) only slightly inhibited by 2–4 dinitrophenol or reduced oxygen tension. The rate of iodide transfer from $I.I.C._1$ into $I.I.C._2$ is inversely related to $I.I.C._1$ iodide concentration. Thus, in high-iodide media the iodide cannot enter $I.I.C._2$ and is thereby excluded from organic binding, even though iodide originally in $I.I.C._2$ is organically bound at a normal rate.

The biochemistry of $I.I.C._2$ as an "activated" form of iodide ion necessary to its subsequent organic binding is under study. This, and the assay applications of these experiments are presented.

20. Studies of the Fractional Rate of Release of Hormonal Iodine From the Thyroid Gland.

David H. Solomon, M.D. (introduced by Marvin S. Levy, M.D.)

Dept. of Medicine, University of California Medical Center, and Research Service and Radioisotope Unit, Veterans Administration Center, Los Angeles, Calif.

Ten patients were allowed to incorporate I^{131} into the thyroid gland. Thereafter further uptake was blocked by methimazole and the thyroid region was counted for three to six weeks. The negative slope of the semi-logarithmic relation of thyroid counts to time equals the net *fractional* rate of release (F.R.R.) of hormonal iodine and is proportional to the daily secretion of hormone divided by the quantity of hormone in the gland.

The administration of methimazole greatly increased the F.R.R. in hyperthyroid patients, but affected it variably in euthyroid persons. The F.R.R. was not further increased by prolonged treatment with methimazole, indicating that the daily amount of hormone secreted fell in proportion to the decreased store of hormone remaining in the gland. The F.R.R. was decreased by Lugol's solution in some, but not all, hyperthyroid patients; the decrease was as great in one patient when euthyroid as when he had been hyperthyroid.

Thyrotropin sharply increased the F.R.R. in a euthryoid and in a hypopituitary patient. The increase in F.R.R. caused by thyrotropin was slightly reduced by added corticotropin. Large doses of estrogen did not alter the F.R.R. of a male hyperthyroid patient. The effect of minor surgical operations in 3 euthyroid persons was variable.

21. *In Vivo* Interconversion of Thyroxine and Triiodothyronine.

John R. Hogness, M.D., Paul Van Arsdel, Jr., M.D. (by invitation) and *Robert H. Williams, M.D.*

Dept. of Medicine, University of Washington School of Medicine, Seattle, Wash.

In view of recent work concerning the relative rates of action of thyroxine and triiodothyronine, some investigators have postulated that triiodothyronine is the active form of the thyroid hormone at the tissue level. With this in mind an experiment was designed in an attempt to demonstrate an interconversion of these substances at the cell level. Two groups of rats were injected intravenously with I^{131}-labeled thyroxine and triiodothyronine, respectively. At time intervals varying from one-half to twenty-four hours, specimens of liver, kidney and muscle were removed, homogenized, digested with trypsin, extracted with butanol and passed through kieselguhr chromatographic columns according to the method of Gross and Pitt-Rivers. In animals given thyroxine, no triiodothyronine was isolated from the tissues assayed; and in animals given triiodothyronine, no thyroxine was recovered. These same results were obtained when thyroidectomized rats were used or when the materials were injected into newborn rats and the entire carcass homogenized, extracted and assayed. These observations cast serious doubt on the hypothesis that thyroxine is converted to triiodothyronine at the tissue level.

22. Studies on the Thyroxine-Protein Complex of Serum.

Jacob Robbins, M.D. (by invitation) and *J. E. Rall, M.D.*

The Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Thyroxine in normal serum is bound largely to a specific protein carrier, with an

electrophoretic mobility intermediate between alpha-1 and alpha-2 globulin (barbital buffer, pH 8.6). The effects of certain compounds, related to thyroxine, on this thyroxine-protein complex were studied with the aid of I^{131}-labeled *l*-thyroxine and filter paper electrophoresis. When diiodotyrosine or diiodothyronine were added to serum in amounts equivalent, on a molar basis, to 50 micrograms of thyroxine per ml., the distribution of I^{131} among the serum protein fractions was essentially unchanged. Triiodothyronine, however, was capable of displacing thyroxine from the specific protein, the radioactivity shifting to all of the other protein fractions, although chiefly to albumin. This displacement was similar to that produced by increments of thyroxine itself, but the molar concentration of triiodothyronine required for this effect was greater than in the case of thyroxine. The lowest concentration of thyroxine at which a shift in its distribution was detected is an index of the binding capacity of the specific protein for thyroxine. In 5 normal serums, this was found to be approximately two to three times the normal serum thyroxine content. The effects of a series of thyroxine analogues on the thyroxine-protein complex is described.

23. The Diphasic Character of Thyroidal I^{131} Clearance.

John H. Nodine, M.D. (by invitation), *William H. Perloff, M.D., Danilo de Albuquerque, M.D.* (by invitation), *Leon Perczek, M.D.* (by invitation) and *Bertram Channick, M.D.* (by invitation).

The Endocrine Dept. of Temple University School of Medicine, and the Endocrine Clinics of the Philadelphia General Hospital, Philadelphia, Pa.

Direct measurements of thyroidal, serum and urinary radioactivity were made in normal individuals ½, 1, 2, 3, 6, 12, 24, 48, 72 and 96 hours after the intravenous administration of 200 microcuries of I^{131}. From these data the thyroidal iodine clearance for each time interval was calculated. In 13 of 15 normal individuals, the clearance was diphasic. The first phase lasted for approximately three hours with a mean clearance value of 1 liter per hour. During the second phase, the mean clearance value was 0.6 liter per hour. The change in the thyroidal I^{131} clearance coincided with alterations in both the disappearance rate from blood and the urinary excretion rates. It is suggested that during the first phase, the major reaction is the equilibration of thyroidal cellular radioiodide with serum radioiodide, whereas during the second phase the limiting factor becomes the rate of hormonal synthesis.

24. Depression of the Thyroidal Iodine Uptake by Hypothalmic Lesions.

W. F. Ganong, M.D., D. S. Fredrickson, M.D. and *D. M. Hume, M.D.* (introduced by Ernest Knobil, Ph.D.)

The Laboratory for Surgical Research, Harvard Medical School, Boston, Mass.

Electrolytic hypothalamic lesions were produced in 16 dogs, and one month later their thyroidal uptake of radioactive iodine was determined by an *in vivo* technique developed in this laboratory. By this method, normal dogs take up 32.0 ± 2.96 per cent of an injected dose of I^{131} in seventy-two hours, whereas hypophysectomized animals take up 3.5 ± 1.34 per cent. Eleven of the dogs with lesions had normal uptakes, and 5 had uptakes in the hypophysectomized range. The area common to the lesions in the latter animals was in, and just above, the anterior end of the median eminence. Lesions elsewhere, including the rest of the median eminence, were ineffective. Pituitary scarring

was slight in all dogs, and uncorrelated with thyroidal uptake. At least some portal-hypophyseal vessels remained intact in all cases.

None of the dogs had adrenocortical atrophy. Four of the dogs with low uptakes had no stress responses (no eosinopenia after trauma and no compensatory adrenal hypertrophy), and 1 had normal adrenocortical responsiveness. Three showed gonadal atrophy, and 2 had normal gonads. Seven of the 11 dogs with normal uptake showed no stress responses and 4 had gonadal atrophy. It is suggested, therefore, that selective thyroid depression can be produced by appropriate hypothalamic lesions in dogs.

FRIDAY, JUNE 18, 1954

9:00 A.M. ADRENALS, ADRENAL HORMONES AND ADRENOCORTICOTROPINS

CHAIRMEN: THOMAS F. GALLAGHER AND FRANK H. TYLER

25. The Diurnal Variation of 17-Hydroxycorticosteroid Levels in Plasma.

Frank H. Tyler, M.D., Claude Migeon, M.D. (by invitation), *A. A. Florentin, M.D.* (by invitation) and *Leo T. Samuels, Ph.D.*

University of Utah, Salt Lake City, Utah.

We have reported previously that plasma levels of 17-hydroxycorticosteroids are higher at 8 A.M. than at subsequent times during the day. This study has been extended to include the entire twenty-four hours. The resulting curve shows that the levels reach their maximum in the early morning, usually at 6 A.M., when average values of 19 μg./100 ml. of plasma have been observed. The levels fall rapidly until noon. They continue to fall slowly until 10 P.M., when minimum values of 6 μg./100 ml. were observed. During the night little change was observed until after 2 P.M., when a rapid rise to the early morning levels ensued.

Preliminary observations in 4 individuals whose pattern of activity and sleep were reversed showed the same type of curve, with maximal elevations at 6 A.M. toward the end of their hours of activity. These studies are being extended to include the determination of eosinophils and serum iron levels, the diurnal variations of which have been reported previously to vary with the activity of the individual.

These observations are important to the understanding of the normal control of adrenal secretion and metabolism of steroids. These and other implications of the data are discussed.

26. Correlation of Diurnal Variations in Eosinophils and 17-Hydroxycorticosteroids in Plasma and Urine.

Richard P. Doe, M.D. (by invitation), *Edmund B. Flink, M.D.* and *Marilyn Gay Flint, B.S.* (by invitation).

The Dept. of Medicine of the Veterans Administration Hospital, and the University of Minnesota, Minneapolis, Minn.

It has been demonstrated that there is a significant diurnal variation in the number of eosinophils (*Journal-Lancet* **71**: 312). In the present study, eosinophils, plasma 17-hydroxycorticosteroids and urine 17-hydroxycorticosteroids were measured four times during twenty-four hours. Results are given as relative values in terms of the percentage of the mean for the 24-hour periods.

Relative Values in 4 Normal Males:

	6 A.M.	9 A.M.	3 P.M.	9 P.M.
Eosinophils	121%	81%	88%	108%
Plasma 17-hydroxycorticosteroids	98%	138%	102%	59%
Urine 17-hydroxycorticosteroids*	47%	192%	104%	57%

* Urines collected from midnight to 6 A.M.; 6 A.M. to noon; noon to 6 P.M.; 6 P.M. to midnight, respectively.

In a group of 13 normal males eosinophil and urine 17-hydroxycorticosteroid values followed a similar pattern. A study was also carried out on 2 normal males, with sampling every three hours. The values for 17-hydroxycorticosteroids were very low and the eosinophils high at midnight and at 3 A.M. Sampling eight times a day tended to bring out the magnitude of the diurnal variation, which is obscured partly by less frequent sampling. The fact that the highest plasma and urine 17-hydroxycorticosteroid values and the lowest eosinophil counts were consistently found in the morning suggests that there is a diurnal variation in adrenocortical activity in normal individuals.

27. Functional Diagnosis of Different States of Adrenocortical Hyperactivity.

R. Burnett, M.D. (by invitation), *M. Rukes, M.D.* (by invitation), *F. Kolb, M.D.* and *P. H. Forsham, M.D.*

The Metabolic Unit, University of California School of Medicine, San Francisco, Calif.

Clinical manifestations of adrenocortical over-activity are frequently variable and inconclusive; however, estimation of total 24-hour urinary 17-hydroxycorticoids, by a modification of the method of Reddy *et al.*, affords the most direct means of evaluating adrenocortical activity. By this method adrenocortical function was studied, with and without ACTH stimulation, in 6 patients with Cushing's syndrome. The following generalizations seem justified: Control 24-hour 17-hydroxycorticoids are generally elevated but do not always exceed high normal levels. In a standard ACTH test, a rise above 50 mg. of 17-hydroxycorticoids separates patients with adrenocortical hyperfunction from normal persons, excepting those previously subjected to stress or having certain types of hepatic insufficiency. In functional adenomas, control values are characteristically variable and the ACTH response is above normal. Patients with adrenocortical carcinomas, however, though having high control 17-hydroxycorticoids, do not show a rise after ACTH. Following ten days of 100 mg. of cortisone orally, the response to the intravenous ACTH test is decreased in normal subjects, and in patients with bilateral adrenal hyperplasia, but not in those with a functional adenoma or carcinoma. If the 17-ketosteroid excretion is above 30 mg. per day, it strongly suggests an adenocarcinoma. These experiences and presacral air studies are presented.

28. Evaluation of Diagnostic Criteria in Cushing's Syndrome.

Anne C. Carter, M.D., Ephraim Shorr, M.D., Thomas N. Roberts, M.D. (by invitation), *Lawrence S. Sonkin, M.D.* (by invitation), *Richard J. Havel, M.D.* (by invitation) and *Ernest T. Livingstone, M.D.* (by invitation).

The Russell Sage Institute of Pathology, Dept. of Medicine, Cornell University Medical College and The New York Hospital, New York, N. Y.

The usefulness for the diagnosis of Cushing's syndrome of a variety of indices has been evaluated from data obtained with 17 patients. Impairment of carbohydrate metabolism as indicated by glucose and/or insulin tolerance tests was present in all subjects. Spontaneous creatinuria, decreased creatine tolerance or low creatinine index was a constant finding. Of the entire series, 13 had a neutrophilia and lymphopenia, whereas only 4 of 11 patients showed an absolute eosinopenia. Elevation of hemoglobin, R.B.C. or hematocrit was seen in 14 out of 17. Hypertension was present in 13 patients. Skeletal x-ray examination revealed osteoporosis in 10 subjects. Calcium, phosphorus and nitrogen balances were negative in only 3 of 13 patients studied on normally adequate diets. Abnormalities in serum electrolyte levels were present in 5 of 16 subjects. The excretion of 17-ketosteroids was elevated in 8 of 16 patients. Urinary 11-oxysteroids were elevated on at least one determination in 8 of 12 patients. Fluctuations in steroid excretion were striking in some subjects. Vaginal smears showed a variety of patterns. The results of the Soffer, calcium tolerance, ACTH and cortisone tests are discussed.

29. Metabolic Pattern Following Alleviation of Cushing's Syndrome.

Philip H. Henneman, M.D., Anne P. Forbes, M.D., John W. Raker, M.D. (by invitation) and *Fuller Albright, M.D.*

The Dept. of Medicine of the Harvard Medical School, and the Medical Service of the Massachusetts General Hospital, Boston, Mass.

To clarify the nature and magnitude of the metabolic deficiencies in Cushing's syndrome the pattern of postoperative repair was studied in a 31-year-old woman with Cushing's syndrome due to an adrenocortical adenoma. Complete metabolic balances for nitrogen, calcium, phosphorus, sodium, potassium, chloride, and magnesium were determined during the twelve days preoperatively and the seventy-eight days postoperatively.

During the preoperative control period the patient achieved nitrogen and phosphorus equilibrium but demonstrated hypercalciuria and a negative calcium balance.

Cortisone was administered for ten days postoperatively.

On discontinuing cortisone the patient had a strongly positive nitrogen and phosphorus balance but demonstrated increasing hypercalciuria, hypercalcemia, and hyperphosphatemia.

On the seventeenth postoperative day the patient began to note arthralgia, marked desquamation of the skin (especially of the face), weakness, fatigue, muscular tenderness, and progressive anorexia and nausea. For ten days the patient was unable to eat; during this period numerous new red striae appeared on the buttocks and the urinary calcium excretion rose to 709 mg. per day.

Following institution of 20 milligrams of cortisone daily on the forty-first postoperative day, the patient's clinical status rapidly improved, the nitrogen and phosphorus balance once more became strongly positive, and the urinary calcium excretion fell to very low levels.

The modus operandi is discussed.

30. Attempt at Classification of Types of Hirsutism in Women by Chromatographic Analysis of the Urinary 17-Ketosteroids.

Raymond Van de Wiele (by invitation), *Joseph W. Jailer, Jay J. Gold* (by invitation) and *Seymour Lieberman* (by invitation).

The College of Physicians and Surgeons, Columbia University, and The Presbyterian Hospital, New York, N. Y.

Correlation between clinical studies and 17-ketosteroid excretion patterns as determined by the gradient elution method and identification by means of infrared spectrophotometry in 25 selected young women has suggested the following classification of hirsutism in women who have no demonstrable ovarian or adrenal tumors, and in whom hirsutism is the primary complaint:

I. Constitutional—Normal ovarian and adrenal function accompanied by normal 17-KS values and chromatographic pattern.

II. Adrenal origin—Normal luteal function in the majority, and elevated 17-KS. Two patterns are found: one with an increased percentage over normal of dehydroisoandrosterone; the other with all fractions of 17-KS elevated. The administration of cortisone to these patients results in a fall in the total and individual 17-KS excretion.

III. Ovarian origin—Menstrual disturbances, with and without the "Stein-Leventhal" type of ovaries. The 17-KS values and chromatographic pattern are normal. There is no clinical response to cortisone administration, and a return of ovulatory menstrual function occurs in most cases after wedge resection. There is no change in the 17-KS chromatograms with the return of normal menstrual function.

IV. Hilar-cell tumors of the ovary have been described as a cause of hirsutism in women but no such instance was encountered in this series.

31. Steroid Isolation Studies in Female Hirsutism: Evidence for Adrenocortical Dysfunction.

Attallah Kappas, M.D. (by invitation), *Olof H. Pearson, M.D., Charles D. West, M.D., Ph.D.* and *T. F. Gallagher, Ph.D.*

The Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Certain adult females in whom clear biochemical evidence of adrenal disorder is unobtainable, nevertheless present stigmata of virilization. Detailed steroid analyses and balance studies were made on such a subject during a control period and ACTH, chorionic gonadotropin and cortisone periods. This patient was a 24-year old female with an eight-year history of progressive hirsutism. Menstrual cycles were ovulatory, regular, normal in duration and unchanged since menarche. Breast development was normal. Body and facial hair was excessive. Pubic hair distribution was masculine. Acne and moderate clitoral enlargement were present. Abdominal exploration revealed normal adrenals and ovaries. Biopsy showed normal adrenals.

Isolation and characterization of individual urinary steroids established abnormally high levels of C_{19}-11 desoxysteroids, notably androsterone and etiocholanolone. The excretion of 17α-hydroxypregnanolone was increased. Individual C_{19}-11 oxysteroids were normal. This steroidal abnormality is reminiscent of that observed in frank virilizing adrenal disease. Chorionic gonadotropin failed to influence urinary ketosteroid levels; cortisone promptly decreased them.

These findings and this clinical picture are interpreted as suggesting a form of benign disease, adrenal in origin, and representing a transitional variant of the adrenogenital syndrome. Details of the steroid isolation and balance studies during experimental periods are reported and discussed in relation to the nature of the adrenal defect in this syndrome.

32. Partial Characterization of C-21 Steroids in the Urine of Patients With the Adrenogenital Syndrome.

Alfred M. Bongiovanni, M.D.

Dept. of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Md.

Previous studies of the 17-hydroxycorticoids in blood have indicated that patients with the adrenogenital syndrome due to adrenocortical hyperplasia fail to synthesize compound F normally. This conclusion was based on low levels of blood 17-hydroxycorticoids and the lack of a rise in response to adrenocorticotropin. Nevertheless, most standard methods have failed to demonstrate abnormally low levels of urinary corticoids. This apparent paradox was the basis for the present investigations. Aliquots of urine from patients with the syndrome and from normal individuals were hydrolyzed with glucuronidase. The extracts were chromatographed on Florosil according to the method of Nelson and the third fraction, eluted with 25 per cent methanol in chloroform, was examined by a variety of tests. There was no difference in the quantities of formaldehyde generated during periodate oxidation. The urinary level of corticoids as determined by the Porter-Silber, and by the Heard and Sobel reducing and ultraviolet absorption (240 mμ) methods revealed no significant diminution of corticoids in the urine of individuals with this condition. The acetaldehydogenic corticoids, however, were markedly elevated. Upon application of the 2,4-dinitrophenylhydrazine reagent to this fraction, there appeared to be a qualitative and quantitative difference between the urines of normal adults and patients with the syndrome. With the latter technique, the results indicated that there was significantly less material resembling compounds E or F in the urine of patients. Speculations are presented as to the possible structure of the C-21 steroids synthesized by patients with the adrenogenital syndrome.

33. Measurement of Dehydroisoandrosterone and Androsterone-Etiocholanolone in Peripheral Plasma of Man.

Claude J. Migeon, M.D. (introduced by Leo T. Samuels, Ph.D.).

The Dept. of Biological Chemistry, University of Utah College of Medicine, Salt Lake City, Utah.

A method for the measurement of dehydroisoandrosterone (DHIA) and androsterone-etiocholanolone in peripheral plasma has been devised. The specificity of the technique has been proved by the isolation and identification of DHIA. The procedure consists of the following steps: a 48-hour continuous extraction with ether at pH 0.5, washing with $NaHCO_3$, chromatography on a Florosil column, removal of phenols with 1N NaOH, paper chromatography with the Bush light petroleum methanol:water (85:15) system, paper elution and micro-Zimmermann reaction on the eluate.

In normal adults, the concentration of DHIA was found to be 30–70 μg. per 100 ml. of plasma, and that of androsterone-etiocholanolone, 5–25 μg. per 100 ml. In most instances, the ratio of DHIA to androsterone-etiocholanolone was greater than 2.

The plasma 17-ketosteroid levels have also been studied following intravenous infusion of ACTH and in cases of hyperadrenocorticism.

34. Status of Patients Treated Continuously with Corticotropin for From Three and One-Half Years to Over Five Years.

William Q. Wolfson, M.D., Clarence Cohn, M.D. (by invitation), *Joseph Kennedy,*

M.D. (by invitation), *Robert Levy, M.D.* (by invitation) and *Leon Lewis, M.D.* by invitation).

The Unit for Metabolic Research, Dept. of Medicine, Wayne University College of Medicine and City of Detroit Receiving Hospital, Detroit; the Dept of Biochemistry and the Division of Medicine, Michael Reese Hospital, Chicago; the Dept. of Medicine, Highland-Alameda County Hospital, Oakland, California; and the Dept. of Obstetrics and Gynecology, Passaic General Hospital, Passaic, N. J.

Between March 1949 and August 1950, continuous long-term treatment with long-acting corticotropin was instituted in 2 gout patients, 4 rheumatoid arthritis patients, 2 patients with chronic chorioretinitis, and 1 moribund patient with acute hemorrhagic disseminated lupus erythematosus. The patient with disseminated lupus has now completed five years of treatment, is married and works an eight-hour day. The 2 patients with chronic chorioretinitis have 20/20 vision and are being maintained on single weekly doses of 80 units of long-acting corticotropin. In 1 gout patient, treatment became unnecessary and was discontinued after two years. The other gout patient who, despite continuous colchicinization to the limit of tolerance, formerly spent months in bed each year, has not lost one day from work. Addition of small amounts of butazolidin has lowered corticotropin requirements enough to control mild hypertension. One patient with rheumatoid arthritis was taken off treatment because of a corticotropin shortage in the autumn of 1949. The other 3 are well and active, although, in one a left ankle proved troublesome until butazolidin was added. This patient and the patient with disseminated lupus are the only persons known to have completed five years of treatment, either with cortisone or corticotropin. The problems arising during treatment are discussed. In selected patients with corticoid-sensitive disease, continuous corticotropin treatment appears suitable for induction of indefinitely prolonged remissions.

35. Effects of Administration of Adrenocorticotropic Hormone and Cortisone During Pregnancy Upon Mothers, Developing Fetuses and Infants.

R. R. Margulis, M.D., C. P. Hodgkinson, M.D., P. J. Howard, M.D. (by invitation) and *E. I. Gordon, M.A.* (by invitation).

Henry Ford Hospital, Detroit, Michigan.

Thirty pregnant women received ACTH and cortisone for various indications during their gestation, in amounts up to 8,000 mg. of cortisone, and 6,500 units of ACTH. This was given over a period ranging from seven days to the entire period of gestation. Eosinophil counts, and determinations of plasma sodium, potassium, chlorides, blood sugar and uric acid were obtained. Body weight, blood pressure and urine analysis were checked weekly. Observations on the clinical and laboratory changes associated with ACTH and cortisone demonstrated insignificant alterations, as reported previously.

A. Immediate and long-term effects on products of gestation were carefully observed. No instances of miscarriages, premature labor, post-maturity or toxemia were encountered.

B. There were 5 stillbirths in patients who had histories of previous stillbirths due to erythroblastosis fetalis. The fetuses in this group, aside from changes characteristic of erythroblastosis fetalis, showed normal development.

C. All infants were watched for immediate post-delivery signs of adrenocortical insufficiency. Only one had suggestive signs, which are discussed. Somatic growth and de-

velopment were followed at frequent intervals and appeared to fall within normal limits. The mental development was followed according to the Catell Intelligence Scale. Results of these psychologic observations were not uniform and warrant discussion.

2:30 P.M. ADRENALS, ADRENAL HORMONES AND ADRENOCORTICOTROPINS

CHAIRMEN: RALPH I. DORFMAN AND JOHN A. LUETSCHER, JR.

36. The Steroid, 11-β-Hydroxylase.

Mika Hayano, Ph.D. (by invitation) and *Ralph I. Dorfman, Ph.D.*

Worcester Foundation for Experimental Biology, Shrewsbury, Mass.

The enzymatic mechanism of the hydroxylation of desoxycorticosterone at the C-11-β-position has been under investigation in the past year. A hypothesis, proposed in an earlier paper, involving a dehydrogenation of the steroid molecule at carbons 9–11 or 11–12, followed by an asymmetric addition of the elements of water, has been tested in two ways: first, with the use of $\Delta^{9(11)}$anhydrocorticosterone and $\Delta^{9(11)}$-anhydrocortisol acetates; and second, with the use of heavy water. The incubation of both the 9(11)-unsaturated compounds with beef adrenal homogenates under a variety of conditions did not yield any significant amounts of corticosterone or cortisol. The synthesis of cortisol from 11-desoxycortisol in the presence of 11-β-hydroxylase in an incubation medium containing heavy water has been accomplished. Analysis of the product after equilibration with methanol and water showed that no deuterium was incorporated into a stable position on the molecule. Both these findings strongly indicate that this stepwise series is not the actual biologic route of synthesis.

An extract of an acetone powder preparation of adrenal mitochondrial fractions containing 11-β-hydroxylase activity has been prepared. TPN (triphosphopyridine nucleotide), fumarate, and oxygen are absolutely necessary for the transformation. Neither high energy phosphate nor inorganic phosphate are required for the reaction.

37. Metabolism and Excretion of Intravenously Administered 4-C^{14}-17-Hydroxycorticosterone.

John E. Plager, Ph.D. (by invitation), *Frank H. Tyler, M.D., Hans H. Hecht, M.D.* (by invitation) and *Leo T. Samuels, Ph.D.*

University of Utah, Salt LakeCity, Utah.

A dose of 420 μg. of 4-C^{14}-17-hydroxycorticosterone (compound F) with 5 μc. of radioactivity was injected intravenously into a 24-year-old normal male subject. Catheters were inserted into the hepatic vein, the femoral artery, and the antecubital vein, and 20-ml. blood samples were removed simultaneously from these catheters fifteen minutes, thirty minutes, one hour, two hours and four hours after injection of the labeled steroid. Urine was collected at regular intervals for the following two-day period. Feces samples were also collected.

Differences between levels of radioactivity in arterial and hepatic vein plasmas indicated a removal of the steroid from circulation by the liver. Biliary excretion of part of this removed material is suggested by the appearance of 0.33 μc. of radioactivity in the feces within forty-eight hours after the compound F was administered. Of the administered radioactivity, 3.7 μc appeared in the urine within this 48-hour period. This excretion followed a logarithmic decay curve which gave the labeled steroid a six-hour half-life in the body.

Identification of the form in which the label appears in the various fractions is now in progress.

38. Diagnosis of Hyperadrenocorticism.

John C. Laidlaw, M.D. (by invitation), *Dalton Jenkins, M.D.* (by invitation), *William J. Reddy, A.B.* (by invitation), *J. Hartwell Harrison, M.D.* (by invitation) and *George W. Thorn, M.D.*

The Dept. of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

The clinical picture of hyperadrenocorticism extends in a broad spectrum from Cushing's syndrome at one end to the adrenogenital syndrome at the other. When the patient presents one or more elements of Cushing's syndrome, attention is focused on the adrenal cortex wherein may be found bilateral hyperplasia, adenoma or carcinoma. These lesions may be differentiated by the response of the urinary 17-hydroxycorticoids to ACTH. Patients with bilateral hyperplasia show a hyperactive response to ACTH; those with carcinoma do not respond. In 3 patients with adrenocortical adenoma, a normal response to ACTH was obtained; a fourth showed a hyperactive response. The degree of autonomy of the adenoma probably determines the response of the tumor to ACTH stimulation.

In contrast to Cushing's syndrome, the virilization syndrome may be caused by a lesion in either the adrenal cortex or the ovary. In such cases, a course of cortisone administration is instituted. If a dramatic fall in urinary 17-ketosteroids occurs, the causative lesion is presumed to be bilateral adrenal hyperplasia. In the absence of depression of 17-ketosteroid excretion, the lesion is considered to be either an adrenocortical tumor or a lesion in the ovary. In these instances, pelvic laporotomy is first performed. In the absence of a responsible ovarian lesion, the adrenals are then explored.

39. New Method for the Determination of ACTH in Blood.

D. H. Nelson and *D. M. Hume* (by invitation).

Naval Medical School and Naval Medical Research Institute, Bethesda, Md.

A method has been developed for the determination of milliunit quantities of ACTH in blood. Hypophysectomized dogs are maintained on ACTH until sixteen hours before they are used for assay. An adrenal vein cannulation is then carried out by a new technique which permits repetitive sampling of adrenal venous blood. These samples are analyzed for corticosteroids. The control collections invariably fail to contain detectable corticosteroids. Single intravenous injections of ACTH peripherally, in doses of 2.5 to 15 milliunits, produce an immediate outpouring of corticosteroids in the adrenal venous blood. This occurs over a 15-minute period following the injection, and the level of corticosteroids returns to zero after this time. Thus a number of test doses and standards can be given successively to the same dog during one experimental period. For the assay of ACTH in the blood of dogs, the blood from the test animal can be injected directly into the assay animal without any preliminary extraction or purification. Peripheral blood from the adrenalectomized dog was found to contain 10–15 milliunits of ACTH per 100 ml. No ACTH has yet been demonstrated in 100-ml. samples of peripheral blood from the normal non-stressed dog.

40. Adrenocorticotropic Substances From Human Placenta.

N. S. Assali, M.D. (by invitation) and *J. Hamermesz, M.D.* (by invitation) (introduced by Charles H. Sawyer).

The Dept. of Obstetrics, University of Cincinnati, and the Dept. of Obstetrics and Gynecology, University of California at Los Angeles, Calif.

The adrenocorticotropic activities of four types of placental extract were studied by Sayer's ascorbic-acid depletion method in hypophysectomized rats. Extract A was prepared by the acetic acid-acetone technique from blood contained in the intervillous space, and extract B by the Astwood technique from the same material. Extracts C and D were prepared from the tissue of chorionic villi by the acetic acid-acetone and Astwood techniques, respectively. Extract D showed a striking adrenocorticotropic activity. A dose-response relationship derived from a study employing 80 animals revealed that 0.1 mg. produces an average adrenal ascorbic-acid depletion of 25 per cent; 1 mg. induces 52 per cent depletion; higher doses stimulate slightly greater responses, and the curve tends to be an hyperbola. Extract B showed some activity but much less than that of D, and the other two extracts were inactive. Comparison with the standard U.S.P corticotropin revealed that the activity of 1 mg. of extract D was approximately equivalent to that of 1 m. u. of the standard. Adrenal maintenance tests were also carried out with extract D in hypophysectomized rats, and the results corroborate those with the ascorbic-acid depletion method. It is concluded that the human placenta produces adrenocorticotropic substances and that the chorionic villi are the probable site of production of these substances.

41. Corticosteroid Secretion Following Hypophysectomy.

Max L. Sweat, Ph.D. and *Gordon L. Farrell, M.D.* (by invitation).

The Dept. of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio.

Adrenal venous blood samples were collected from dogs, extracted with chloroform, partitioned between petroleum ether and 70 per cent ethanol, chromatographed on silica gel, and analyzed by the fluorescent method of Sweat and the phenylhydrazine reaction of Porter and Silber. After hypophysectomy the rate of secretion of corticosterone (B) and 17-hydroxycorticosterone (F) decreases rapidly. At the end of one hour the amount of F secreted per minute is approximately one half the rate of secretion by the adrenals before hypophysectomy. At the end of two hours the quantity secreted per minute is approximately one third to one tenth the prehypophysectomy rate. The rate of secretion of F appears to remain constant between the interval of two and four hours after hypophysectomy in most animals studied. The rate of secretion of B decreases after hypophysectomy in a pattern similar to that of F. Data on the hypophysectomized rat are also presented.

42. Chlorohydrocortisone Acetate, A Highly Potent Derivative of Compound F.

Alan Goldfien, M.D. (by invitation), *Paul M. Beigelman, M.D.* (by invitation), *John C. Laidlaw, M.D.* (by invitation) and *George W. Thorn, M.D.*

The Dept. of Medicine, Harvard Medical School and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Mass.

9-Alpha-chlorohydrocortisone acetate has been synthesized by Fried and Sabo (*J. Am. Chem. Soc.* **75**: 2273, 1953) and has been found to be more highly active than cortisone acetate in its effect on glycogen deposition, thymus involution and sodium retention in rats. We have compared the potency of this compound with hydrocortisone acetate in a variety of metabolic and clinical studies in both addisonian and nonaddisonian

patients. Qualitatively, the effects of the two compounds are identical. In doses of 50–100 mgs. by mouth, however, chlorohydrocortisone acetate is considerably more potent than hydrocortisone acetate in its influence on circulating eosinophils and on the renal excretion of sodium, potassium, chloride and uric acid. The sodium chloride retention and potassium diuresis observed after a single oral dose of chlorohydrocortisone acetate is particularly marked. Moreover this compound is at least as effective as hydrocortisone acetate in restoring to normal the impaired capacity of the addisonian patient to excrete an ingested water load. As is the case with hydrocortisone acetate, the new compound is a weakly potent precursor of urinary 17-ketosteroids. Studies of the use of chlorohydrocortisone acetate as the sole maintenance therapy in Addison's disease and of its effect on patients with virilization due to bilateral adrenocortical hyperplasia are presented.

43. Factors Regulating the Output of Sodium-Retaining Corticoid of Human Urine.

B. J. Axelrad, M.D. (by invitation), *B. B. Johnson, M.D.* (by invitation) and *J. A. Luetscher, Jr., M.D.*

The Dept. of Medicine, Stanford University School of Medicine, San Francisco, Calif.

A highly active sodium-retaining corticoid, which appears to be identical with electrocortin, has been extracted from normal human urine. The active material is separated from other urinary steroids by paper chromatography and is quantitatively assayed in adrenalectomized rats.

Normal children and adults excrete small amounts of sodium-retaining corticoid, which is measurable only when the urine is subjected to prolonged acid hydrolysis and when large doses of urine extract are bioassayed.

When the dietary sodium intake of normal men is restricted, an increased output of sodium-retaining corticoid appears in the urine as the sodium concentration falls.

Through the courtesy of Drs. Forsham, Barthold, DiRaimondo, and Orr, the effect of a single, large dose of ACTH gel in normal men has been studied. No significant increase of sodium-retaining corticoid was observed.

The greatly increased output of sodium-retaining corticoid in the nephrotic syndrome is reduced during or after administration of cortisone, hydrocortisone, or ACTH when diuresis increases. When diuresis fails, the reduction is slight or absent.

Thus, the output of sodium-retaining corticoid may be increased by sodium deprivation and by certain diseases, with marked reduction of urinary sodium, but the output is not stimulated by ACTH.

44. Studies of Electrocortin.

Robert Gaunt, Ph.D., A. S. Gordon, Ph.D., A. A. Renzi, Ph.D. (by invitation), *J. Padawer, Ph.D.* (by invitation), *G. J. Fruhman, M.S.* (by invitation) and *Martha Gilman, M.S.* (by invitation).

The Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J., and the Dept. of Biology, New York University, New York, N. Y.

Experiments are in progress designed to characterize the biologic actions of electrocortin, the highly potent sodium-retaining compound crystallized from adrenocortical extracts by Simpson, Tait, Wettstein, Neher, v. Euw and Reichstein (*Experimentia* **9**: 33, 1953). Since only microgram quantities of the substance have been available (supplied

by Dr. A. Wettstein, Ciba, Ltd., Basle), the initial tests were essentially qualitative rather than quantitative.

Electrocortin was used in the Selye-Schenker cold stress test (9 or 10 adrenalectomized rats per group). A dose of 20 μg. of electrocortin in oil prolonged life significantly and to an equal or greater extent than a similar amount of cortisone in two tests: electrocortin, +21 per cent and +46 per cent; cortisone, +15 per cent and +36 per cent. Desoxycorticosterone (20 μg.) showed significant activity in only 1 of 3 such tests.

A dose of 10 μg. of electrocortin in oil caused significant eosinopenia in only 2 of 10 adrenalectomized mice, using the Speirs' assay, whereas 10 μg. of cortisone induced eosinopenia in 9 animals tested (mean drop, 61 per cent). Electrocortin in a dose of 20 μg., however, caused a mean eosinophil drop of 65 per cent in the 3 cases tested. Speirs (personal communication) also reports a cortisone-like action of electrocortin in the eosinophil test. Additional work is reported.

45. Excretion of Sodium-Retaining Substances.

Eleanor H. Venning, Ph.D., A. Carballeira, M.D. (by invitation) and *Inge Dyrenfurth, D.Sc.* (by invitation).

McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Using a bioassay that measures sodium retention in the adrenalectomized rat, we have determined the sodium-retaining effect of urinary extracts obtained from normal individuals following the administration of ACTH and growth hormone, and also from patients with a variety of diseases.

Normal individuals excrete only very small amounts of sodium-retaining substances and frequently the extracts cause slight sodium excretion in the test animal. ACTH has little effect on the excretion of these substances. In our assay the effect of ACTH might be masked by an increase in output of glucocorticoids, as cortisone has the opposite effect of desoxycorticosterone, causing sodium excretion. Following the administration of growth hormone, there is an increase in the sodium-retaining effect in normal individuals.

Increased amounts of these substances have been found in the urine of patients with toxemia of pregnancy, Cushing's disease, adrenal tumor and malignant hypertension.

The hydrolysis of the urine with β-glucuronidase yields increased amounts of sodium-retaining substances.

SATURDAY, JUNE 19, 1954

9:00 A.M. PARATHYROID, PITUITARY AND GROWTH HORMONES

CHAIRMEN: ROBERTO F. ESCAMILLA AND ARTHUR GROLLMAN

46. Endocrinologic Studies on the Nephrectomized Dog.

Arthur Grollman, M.D.

Southwestern Medical School of the University of Texas, Dallas, Texas.

It is possible to maintain the bilaterally nephrectomized dog in good condition for periods of five to ten days without recourse to extrarenal excretory measures and for three or more months when intermittent peritoneal lavage is utilized. Such preparations have been used in a study of a number of problems of endocrinologic interest. It has been demonstrated, for example, that the adrenalectomized and the adrenalectomized-

nephrectomized dog may be maintained for a month or more without recourse to endocrine therapy. The technique when applied to the parathyroidectomized dog has demonstrated that, contrary to a widely prevalent view, the parathyroids exert their effect on the blood calcium level by an extrarenal action. It has also been possible to study the exchange between the cells and extracellular fluid under the influence of various hormones. Cortisone has been demonstrated to exert a marked hypercalcemic effect, which may prove fatal in the nephrectomized animal. The method of peritoneal lavage in the intact as well as in the nephrectomized dog also permits obtaining a solution in equilibrium with the ultrafiltrable constituents of the extracellular fluid, including its diffusible hormones. Technical details and illustrative results are presented.

47. Effects of Benemid (Probenecid) in the Treatment of Hypoparathyroidism and Pseudohypoparathyroidism.

Felix O. Kolb, M.D. and *J. Max Rukes, M.D.* (by invitation).

The Metabolic Unit and the Dept. of Medicine, University of California, School of Medicine, San Francisco, Calif.

We have used Benemid (probenecid) with good results in a series of 8 cases of hypoparathyroidism, as previously suggested by Hoffman *et al.* In gouty subjects Benemid inhibits reversibly tubular reabsorption of uric acid, without affecting urinary phosphorus excretion; the fecal phosphorus, however, is increased. Balance studies in our cases of hypoparathyroidism during Benemid therapy have shown prompt phosphate diuresis with a fall in the serum phosphorus level to normal, and control of symptoms of tetany over prolonged periods. On stopping Benemid the phosphorus returned to pretreatment levels and symptoms recurred. By contrast, the serum calcium concentration rose slowly and reached normal levels only after adding calcium to the regimen. No phosphate diuresis was demonstrated in a case of pseudohypoparathyroidism, by either Benemid or Parathormone. However, the serum phosphorus level fell to normal during Benemid therapy, presumably through a fecal mechanism. The dissociation of action of Benemid on the concentration of serum phosphorus and calcium, and the differences in response of hypo- and pseudohypoparathyroid patients add information to the mechanism of action of parathyroid hormone.

48. Phosphate-Losing Osteomalacia, and Its Paradoxical Relief by Large Doses of Vitamin D.

Russell Fraser (by invitation) and *B. E. C. Nordin* (by invitation) (introduced by Gilbert S. Gordan).

The Postgraduate Medical School of London, England.

Studies have been made on a 33-year-old female dwarf with osteomalacia (triradiate pelvis, Milkman's fractures, low serum phosphate level) confirmed by bone biopsy and attributed to "vitamin D resistance" since there was no steatorrhea or acidosis, no abnormal excretion of amino acids, glucose or albumin in the urine and since the blood urea level was normal, as was the ability to excrete an acid load. Callus formed in the fractures, and the blood and urinary calcium and phosphate rose after high vitamin D dosage. Oral calcium increased the urinary calcium normally. Calcium tolerance tests (intravenous) showed merely a normal retention of calcium, which was but slightly increased by moderate or high vitamin D dosage. The phosphate leak was suppressed acutely during the intravenous calcium test. The findings suggest a primary metabolic disorder of bone calcification.

49. The Fine Structure of the Anterior Pituitary Gland As Revealed by Electron Microscopy.

Marilyn Gist Farquhar, M.A. and *James F. Rinehart, M.D.* (by invitation) (introduced by Roberto F. Escamilla, M.D.).

Dept. of Pathology, University of California School of Medicine, San Francisco, Calif.

New techniques promise to clarify the baffling problem of relating cell structure and function in the anterior pituitary gland. In addition to new selective staining methods, examination of ultra-thin sections of tissue with the electron microscope offers considerable promise. Detail of structure is revealed that can be achieved by no other method. Fine cytoplasmic constituents including mitochondria, Golgi apparatus, "specific" granules, canaliculi and vesicles often may be seen in a single cell. Acidophils contain relatively coarse granules, whereas the granules in basophils are fine. Chromophobes usually retain a scattering of granules, allowing them to be classed in the acidophilic or basophilic series. It is evident that the functional activity of this tissue is great and is reflected in changes of the fine cell structure. The striking cellular alterations occurring after castration in the female rat have been studied in some detail (Farquhar and Rinehart: *Endocrinology*, in press). These observations and current studies in the male castrate are presented. Considerable evidence has accrued recently indicating that gonadotropic and thyrotropic cells, though both basophils, present distinctive structural features. Electron microscopic studies supporting this view are presented. Electron microscopy offers great promise in elucidation of vital problems of cytophysiology.

50. Physiologic Effects of Hypophysectomy in Man.

Charles D. West, M.D., Min C. Li, M.D. (by invitation), *John P. Maclean, M.D.* (by invitation), *Joseph E. Rall, M.D.* and *Olof H. Pearson, M.D.*

The Sloan-Kettering Institute, New York, N. Y.

Hypophysectomy has been performed in 18 patients with advanced cancer in the past two years. Cortisone or ACTH replacement therapy was used during surgery. In 9 patients diabetes insipidus developed within six hours to six days after surgery, requiring pitressin for control. These patients were later shown to have incomplete removal of the pituitary. In 9 patients diabetes insipidus did not develop; in 5 of these, autopsy showed that pituitary removal was complete.

In all patients there was an immediate fall in the blood protein-bound iodine level and in thyroidal I^{131} uptake. In the patients with incomplete hypophysectomy, these indices returned to normal within a few weeks. In 1 patient in whom hypophysectomy appeared to be complete, thyroidal I^{131} uptake remained low for four and a half months and a clinical picture of hypothyroidism developed. Thyroid function was increased in this patient by administration of thyroid-stimulating hormone.

Cortisone maintenance therapy could be withdrawn without ill effects in patients with incomplete hypophysectomy. In the patient with apparent complete hypophysectomy, withdrawal of cortisone resulted in acute adrenal insufficiency within five days.

Follicle-stimulating hormone disappeared from the urine postoperatively and remained absent in patients with complete hypophysectomy.

51. Growth Hormone and Lymphatic Organs.

L. F. Kumagai, M.D. (by invitation), *B. A. Brill, B.A.* (by invitation) and *Thomas F. Dougherty, Ph.D.*

The Dept. of Anatomy, University of Utah, Salt Lake City, Utah.

Intact male mice (CBA) were fasted for forty-eight hours and then were given graded amounts of growth hormone (1.0, 4.0 and 6.0 mg.) for four days. Male rats were fasted for forty-eight hours, then hypophysectomized. Growth hormone (100 and 500 μg.; 1.0, 2.0 and 4.0 mg.) was administered for ten days. A differential response of changes in weights of spleens, thymi and peripheral nodes was observed. Splenic weights of both mice and rats were significantly greater than respective controls, and demonstrated a dose-response relationship. Thymic weights of treated groups were the same or less than their controls. The peripheral node weights of rats increased above their control values in all doses; however, a dose response was not apparent. Increased splenic size was due to increases of both red and white pulp. Extramedullary erythropoiesis was marked. Basophilic and polychromatophilic erythroblasts predominated. Lymphocytes of thymi and lymph nodes of treated animals were larger, and uniform in size as compared to controls. Reticular hyperplasia was not prominent. Intermediately differentiated erythroblasts characterized the bone marrow of the hormone-treated animals. It appears that growth hormone stimulated proliferation but not maturation of erythropoietic cells. The possibility of using splenic weights as a method for assaying growth hormone is discussed.

52. Growth Hormone in Normal and Hypophysectomized Immature Rhesus Monkeys.

E. Knobil, Ph.D., W. F. Ganong, M.D. (by invitation) and *R. O. Greep, Ph.D.*

Harvard Medical School and Harvard School of Dental Medicine, Boston, Mass.

Beef growth hormone (Armour lots 369-265 and R-491017) was administered to intact and hypophysectomized immature rhesus monkeys at a dose of 5 mg. of Armour standard equivalent per kilogram per day. The hormone was administered intramuscularly in aqueous solutions for periods of from nine to fifteen days.

In the intact animals this regimen was without effect on the rate of body-weight gain and the blood concentrations of glucose, nonprotein nitrogen, amino-acid nitrogen and inorganic phosphorus. Glucose tolerance remained unchanged.

In the hypophysectomized monkeys, growth hormone effected a decrease in nonprotein nitrogen concentrations when compared to hypophysectomized control animals. However, the plateau type of growth curve exhibited by these animals was not altered by hormone administration. Blood glucose, inorganic phosphorus and amino-acid nitrogen levels remained unchanged. The lowered hematocrit values seen in the hypophysectomized animals were not corrected by growth hormone.

Other criteria of growth hormone activity are under investigation at present and the effects of prolonged hormone administration are being studied in an effort to shed some light on the nature of the physiologic action of this substance in primates.

53. Inhibition of the Fat-Mobilizing Properties of Ethionine by Anabolic Steroids and Growth Hormone.

Albert Segaloff, M.D. and *Emmanuel Farber, M.D., Ph.D.* (by invitation).

The Depts. of Medicine and Pathology, Tulane University of Louisiana, School of Medicine, and the Endocrine Research Laboratory of the Alton Ochsner Medical Foundation, New Orleans, La.

Ethionine, an antagonistic analogue of methionine, was previously shown to produce fatty livers in fasting female or castrated rats of both sexes. This does not occur in male

rats nor in rats adequately treated with testosterone propionate. This increase in liver lipid is due to neutral fat, presumably mobilized from the depots.

We have now found that Δ^5-androstene-3β,17β, diol, and methyltestosterone are at least as effective as testosterone propionate in preventing this mobilization of fat in the liver.

Hypophyseal growth hormone is considered capable of mobilization of fat to the liver in mice. However, we have found that growth hormone (hog and beef) is effective in preventing the ethionine fatty liver in rats.

Thyroid-stimulating hormone, insulin, desoxycorticosterone acetate, estradiol dipropionate and cortisone acetate all failed to prevent ethionine fatty liver. In fact, some of these agents actually intensified the fat accumulation.

Evidence is available suggesting a relationship between fatty liver and disturbed hepatic protein synthesis in ethionine-treated rats. It appears significant that, of the agents tested, only those capable of producing nitrogen retention prevented the ethionine fatty liver.

54. Antidiuretic Activity of Normal and Hypophysectomized Rats.

Charles W. Lloyd, M.D. and (by invitation) *Sophie Pierog, B.A., Erich H. Loewy, B.A., Karol Bradwick, B.A.* and *Robert Sostheim, B.A.*

State University of New York, College of Medicine, Syracuse, N. Y.

Adult male Sprague-Dawley rats were used throughout. Antidiuretic activity was assayed intravenously in the alcohol-anesthetized, hydrated rat. Serum of normal rats contains antidiuretic activity equivalent to 50 to 400 microunits of pitressin per ml. Posterior lobe contains activity equivalent to about 25 milliunits, and hypothalamus about 5 milliunits of pitressin. Serum of recently hypophysectomized rats contains no detectable activity, *i.e.*, less than 30 microunits of pitressin per ml. The hypothalamus contains less than 5 milliunits of activity. The serum of some animals hypophysectomized for thirty days or more contains essentially the same activity as that found in serum of normal animals. The hypothalamus contains more antidiuretic activity (about 10 m.u) than is present in the recently hypophysectomized animal and at least as much and probably more than in the normal animal. Measures which cause a release of antidiuretic hormone into the blood stream decrease the antidiuretic content of the hypothalamus. These data suggest that a functional reorganization can take place after hypophysectomy.

55. Hereditary Diabetes Insipidus, With Reports of Many Instances in Seven Generations of One Family.

Ernest L. Levinger, M.D. (by invitation) and *Roberto F. Escamilla, M.D.*

The Endocrine Clinic, Division of Medicine, University of California School of Medicine, San Francisco, Calif.

Diabetes insipidus occasionally occurs as a hereditary family characteristic. Relatively few family surveys have appeared so far in the American literature. Starting with one patient, a pedigree has been elicited and investigated of an affected family covering seven generations. Many instances of the disease were found, and analysis suggests autosomal transmission of a dominant gene with an incomplete penetrance. The genetic nature of the transmission is discussed.

The first published family statistics were by La Combe in 1841. Since then many studies of this type have appeared in the German, French, and Scandinavian literatures.

A thorough review was published by Forssman in Sweden in 1945, and a more recent survey of a Canadian family by Pender and Fraser in 1953. We believe that the study here presented represents one of the largest series of cases in one family yet published in this country, and may possibly be one of the larger in world literature.

56. Melanocyte-Stimulating Hormone (Intermedin, Melanophore Hormone) in Human Blood and Urine.

Kazuo Shizume, M.D., Aaron B. Lerner, M.D., Ph.D. and *Thomas B. Fitzpatrick, M.D., Ph.D.* (introduced by Peter H. Forsham, M.D.)

Division of Dermatology, University of Oregon Medical School, Portland, Ore.

A bioassay method for quantitative determination of the melanocyte-stimulating hormone (MSH) has been devised. This method utilizes changes in light reflection from isolated frog skin, before and after immersion in solutions containing MSH. A photoelectric reflection meter is used for the measurements. Within certain limits, the logarithm of MSH concentration is proportional to the ratio of the changes in reflectance obtained with unknown and standard amounts of hormone. By using the proper extraction procedures one can determine MSH in blood and urine. Analysis of 24-hour urine specimens and of blood specimens yielded the following results:

1. Very small but detectable amounts of MSH were found in the blood and urine of most normal males and females.
2. In pregnancy there is increased output of MSH in the urine and an increased level in the blood.
3. Patients with Addison's disease and bilaterally adrenalectomized patients not receiving cortisone showed a high urinary output of MSH and an increased blood level of MSH.
4. Urinary MSH increased after the withdrawal of cortisone in a patient with Addison's disease.

MSH in blood and urine after the intravenous and intramuscular administration of MSH was also studied. About 1 to 4 per cent of the hormone is excreted into the urine. MSH disappears rapidly from the blood stream.

57. Endocrine Control of an Induced Hepatic Enzyme System.

Irving I. Geschwind, Ph.D. and *Choh Hao Li, Ph.D.*

Hormone Research Laboratory, Dept. of Biochemistry, University of California, Berkeley, Calif.

A study has been made of the effect of the endocrine state on both the constitutive and induced levels of the tryptophane peroxidase-oxidase enzyme system.

Hypophysectomy results in a decrease of 35 per cent in the induced enzyme level by the fourth postoperative day, and by the fourteenth day the response has decreased to one third of normal (Geschwind and Li: *Nature* **127**: 732, 1953). Adrenalectomy causes a precipitous fall in the induced level, to 25 per cent of normal within four days after operation. Ten days later this value is still essentially unchanged. Neither operative procedure causes any significant change in the constitutive enzyme level. Thyroidectomy promotes an increase of 174 per cent in the latter level by the fourteenth day after surgery, but produces a decrease in the induced level to 65 per cent of normal.

In hypophysectomized rats, no change from the control value of either enzyme level is observed when the animals are maintained with either growth hormone or thyroxine.

The administration of ACTH to such an animal effects a considerable increase in the constitutive level but results in only a slight increase in the induced activity. 17-Hydroxycorticosterone is capable of elevating the enzymatic response to induction in hypophysectomized or adrenalectomized animals to 70 per cent of normal, but this increase is due, to a large extent, to a marked rise in the constitutive enzyme level.

Other endocrine factors affecting the control of the enzyme activity are discussed.

2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION

CHAIRMEN: RANDALL G. SPRAGUE AND EARL T. ENGLE

58. Hypophysectomy in Patients with Malignant Diabetic Vascular Disease—Metabolic and Clinical Evaluation.

Laurance W. Kinsell, M.D., Lester Lawrence, M.D., Harry E. Balch, M.D. and *Robert D. Weyand, M.D.*

The Dept. of Medicine and Neurosurgery, Samuel Merritt Hospital and the Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, Calif.

Patients with progressive malignant vascular disease in association with the "juvenile type of diabetes" have been studied intensively in this Institute during the past several years. Even under "ideal conditions," *i.e.*, chemically constant dietary intake, constant insulin dosage, and constantly controlled activity, progression of the disease occurs, although probably at a slower rate. Because of this, it was decided to perform total hypophysectomy in a small selected group of such patients to determine whether the amelioration of diabetes known to occur in diabetics following the onset of Sheehan's syndrome, and in experimental animals subjected to hypophysectomy, would be associated with improvement in the vascular disease.

Only patients, in whom a fatal outcome appeared unquestionable (because of progressive renal insufficiency), were selected for this procedure initially. To date the results from both a metabolic and clinical standpoint appear to be encouraging. A summary of the metabolic and clinical changes is presented.

59. Increased Insulin Sensitivity in Rats with Hypothalamic Lesions, and Its Interpretation.

B. N. Spirtos, M.S. (by invitation), *W. R. Ingram, Ph.D.* (by invitation), *E. M. Bogdanove, Ph.D.* (by invitation) and *N. S. Halmi, M.D.*

Dept. of Anatomy, State University of Iowa, Iowa City, Iowa.

Rats bearing electrolytic hypothalamic lesions and fed a restricted pellet diet showed an exaggerated hypoglycemic response to insulin (0.1 unit/Kg. given after twenty-four hours of fasting). Their insulin sensitivity was restored to normal by somatotropin (1 mg./day for five days), but not by cortisone (1 mg./day for five days). The great sensitivity of hypophysectomized rats to insulin was substantially alleviated by somatotropin, whereas cortisone was of benefit only in so far as it elevated the initial blood sugar level. The enhanced insulin sensitivity of adrenalectomized rats, on the other hand, was not influenced by somatotropin, but could be abolished by the administration of cortisone. On the basis of these findings it is suggested that hypothalamic lesions affect the insulin sensitivity of rats by interfering with the secretion of somatotropin. Lesions having this effect do not necessarily impede other functions of the anterior lobe.

60. Some Effects of Growth Hormone on the Structure and Function of the Islets of Langerhans.

Leslie L. Bennett, M.D., Ph.D., James H. R. Sutherland, A.B. (by invitation) and *Adrienne A. Batts, M.D.* (by invitation).

The Dept. of Physiology and The Institute of Experimental Biology, University of California, Berkeley, Calif.

The effects of growth hormone (GH) on the structure and function of the islets of Langerhans were studied by 1) a metabolic-histologic experiment in partially depancreatized, diabetic rats, and 2) the method of pancreatic implants into depancreatized dogs. When GH is given to diabetic rats their glycosuria increases initially and then declines to, or below preinjection levels. In untreated diabetic rats the islets show enlarged cells, loss of specific granulation, hydropic degeneration, and marked fibrosis. In animals treated with GH for six days the cells have sharp borders; they are more numerous, large and filled with homogeneous cytoplasm showing little evidence of injury. New, healthy cells are found in fibrotic islets. These findings are consistent with the metabolic data and support the view that the islets of partially depancreatized rats can produce more insulin under the influence of GH.

In the following experiments at least 4 dogs were used in each group. The pancreas of a normal dog implanted by vascular anastomosis into a pancreatectomized dog with a glycemia of about 300 mg. per 100 ml. will reduce the blood glucose level to normal within three hours and then keep it normal. This reduction corresponds to the effect of an insulin infusion of .08 μ/Kg./hr., which is reduced stepwise to .02 μ/Kg./hr. The administration of GH plus the infusion of insulin at this rate demonstrates, in some dogs, an anti-insulin effect of the GH. GH administration to pancreatectomized dogs with an implanted pancreas does not inhibit the rate at which the pancreas reduces the blood sugar level to normal. These findings indicate that GH does not actively inhibit insulin secretion and may enhance it.

61. Metabolic Adjustments During Stress: The Effect of Intravenous Glucose on Pyruvate During Compound F Infusion.

Thomas F. Frawley, M.D., John J. Garrett, M.D. (by invitation) and *Paul J. Rosch* (by invitation).

The Dept. of Endocrinology and Metabolism, Albany Medical College, and the Endocrine-Metabolic Clinic, Albany Hospital, Albany, N. Y.

Studies are in progress to ascertain whether certain alterations in carbohydrate metabolism during stress are direct or indirect consequences of alterations in adrenocortical activity. This laboratory previously described two patterns of alteration in blood pyruvate in a significant number of patients receiving ACTH or cortisone therapy, often independent of changes in glucose, phosphate and potassium concentrations. Moderate dosages appeared to cause a prolonged elevation of the pyruvate level during the intravenous glucose tolerance test, whereas larger dosages intensified this effect and also increased fasting pyruvate levels. Normal individuals were given constant infusions of compound F (12–25 mg./hr.) for seven hours. At the beginning of the third hour, intravenous glucose (0.5 Gm./Kg. of body weight, as a 20 per cent solution) was administered for twenty-five minutes. Blood sugar and pyruvate levels showed little variation preceding the glucose. Following glucose the blood sugar level did not differ from control values and the pyruvate response was only slightly prolonged with the 25-

mg./hr. dose of compound F. Serum phosphate levels decreased normally. A less marked tendency toward these effects developed with infusions of aqueous adrenocortical extract (equivalent to 3 mg. of compound E per hour).

These studies suggest an adrenal influence at or near the pyruvate level. The more marked changes in glucose and pyruvate observed in various patients treated with adrenal hormones may be the result of the added stress of the specific illness. This is consistent with the concept of the minor but significant role of the adrenal cortex in the metabolic adjustments occurring during stress.

62. Ketogenic Activity of Corticotropin in the Rat.

Frank L. Engel, M.D. and *Mildred G. Engel, M.S.* (by invitation).

Depts. of Medicine and Physiology, Duke University, Durham, N. C.

Previous reports on the ketogenic effect of corticotropin are contradictory. In general, single injections have induced either no change or an increase in ketosis, whereas multiple injections have had no effect or inhibited fasting or stress ketosis. We have reported that cortisone and hydrocortisone inhibited fasting and cold stress ketosis. These observations suggest that corticotropin is contaminated with a factor which induces ketosis rapidly, whereas the steroids secreted in response to corticotropin inhibit ketosis. The ketogenic effect of pituitary preparations was tested by intraperitoneal injection into 18-hour fasted rats under Nembutal anesthesia at a constant environmental temperature of 33° C. for three and a half hours. All samples of corticotropin tested induced ketosis in three and a half hours in normal rats at doses of 1–20 units. The most active preparation was one prepared by the Oxycel method and assaying 33 units/mg. It was effective at a dose of 1 unit (0.03 mg.). Pretreatment with 5 mg. of cortisone acetate for two days inhibited ketosis. Adrenalectomized rats maintained with 0.5–1.0 mg. of cortisone exhibited a ketogenic response to corticotropin but required larger doses than did normal rats. Animals maintained with desoxycorticosterone did not exhibit ketosis after corticotropin. It is concluded that corticotropin has ketogenic activity which is not mediated by the adrenal cortex but is dependent on the presence of adrenal hormone.

READ BY TITLE

GONADS

63. A Routine Bioassay Procedure for Gonadotropic Extracts.

Leon M. Neal, B.S. (by invitation), *Richard C. Barton, M.D.* (by invitation), *Emil G. Holmstrom, M.D.* (by invitation) and *Hilton A. Salhanick, Ph.D.*

The Dept. of Obstetrics and Gynecology, University of Utah, and Memorial Medical Center, Salt Lake City, Utah.

The synergistic effect of follicle-stimulating hormone (FSH) and human chorionic gonadotropin (HCG) on the ovaries has recently been utilized for the bioassay of FSH preparations by Steelman and Pohley (*Endocrinology* **53**: 604, 1953). A similar method was applied to the assay of gonadotropic extracts of urine. Optimal conditions for the assay were defined:

1) The immature mouse is more sensitive than either the immature rat or the hypophysectomized rat.

2) The extract is dissolved in 20–25 per cent polyvinylpyrrolidone, which is the most effective retardant vehicle tested.

3) Forty international units of HCG are injected into each assay animal from this same solution.

4) An injection schedule of twice a day for three days was found to be preferable to other schedules tested.

5) Maximal ovarian response is detected 24–36 hours after the last injection.

This procedure, based upon increase in ovarian weight, is more sensitive than other routine procedures utilizing ovarian and uterine weight response. The effect of added luteinizing hormone (LH) was masked by the chorionic gonadotropin; therefore, greater specificity is obtainable even in the intact immature animal. The greatest advantage is that urine extracts can be compared with a laboratory standard. Typical assay results of urine extracts from normal, castrated, and menopausal women are presented.

64. Reduction of Response to Gonadotropin in Chicks Deficient in Pantothenic Acid or Injected with Squalene.

Irene T. Kline, Ph.D.

The Depts. of Biochemistry and Obstetrics and Gynecology, School of Medicine, Western Reserve University, Cleveland, Ohio.

Synthesis of estrogens and androgens by pantothenic acid-deficient pullets and cockerels in response to pregnant mares' serum gonadotropin (PMSG) is markedly reduced in comparison to that observed in thiamine-deficient chicks or in inanition (body weight) controls. Oviduct weights of pullets and comb weights of both sexes are lowered in pantothenate-deficient chicks by 50 to 90 per cent depending upon the experimental conditions. Some reduction in weight of primary target organs (ovaries and testes) in response to PMSG and of secondary target organs (oviducts and combs) in response to stilbestrol or testosterone propionate occurs in chicks severely deficient in pantothenic acid, but these two defects combined account for only part of the drastic impairment in the over-all response to PMSG.

Attempts to circumvent with squalene the biochemical block which presumably exists in the synthetic mechanism at the acetate level due to an insufficiency of coenzyme A have failed, with the doses of squalene thus far employed (0.1 and 0.2 ml. of S. Q. daily). On the contrary, squalene inhibits the response to both exogenous and endogenous gonadotropin. In chicks fed a synthetic diet, simultaneous injection at different sites of squalene and PMSG (2500 I.U. to 7500 I.U. total dose) can result not only in a response dramatically lower than to PMSG alone, of oviducts and combs, while interfering only moderately with the response of the ovaries or testes of those on a complete diet, but also impairs further the already poor response to PMSG of those deficient in pantothenic acid. In cockerels (1 or 2 weeks old) fed a commercial mash, doses of 0.2 or 0.4 ml. of squalene daily do not interfere with the response to testosterone propionate (0.5 mg. daily) but in cockerels treated for two weeks with squalene alone, comb growth is significantly decreased without any change in testis or body weights in comparison to uninjected controls.

65. Effect of Antihyaluronidase Compounds Upon Gonadotropic Hormone Preparations.

Herbert S. Kupperman, Elizabeth L. Wyant (by invitation), *Arthur C. DeGraff* (by invitation) and *John P. Jenkins* (by invitation).

Department of Therapeutics, New York University-Bellevue Medical Center, New York, N. Y.

Pituitary gonadotropin, (FSH), pregnant mares' serum (PSM) and chorionic gonadotropin (APL) were administered to immature female rats, alone, and together with the following preparations: 5 per cent potassium chondroitin sulfate, 2 per cent phosphorylated hesperidin, 2.5 per cent α-tocopherol phosphate (adjusted to a pH of 8.6 to 8.8), 3 per cent sodium alginate, and 2 per cent hesperidin methyl chalcone. Augmentation of the gonadotropic activity of the FSH preparation was achieved with all the compounds except hesperidin methyl chalcone and sodium alginate. When concentration of the antihyaluronidase compounds was increased, no further enhancement of augmentation was attained. Responses to APL and PMS were not appreciably affected by the addition of antihyaluronidase preparations. Augmentation of FSH was achieved in hypophysectomized animals, indicating that the pituitary played no role in the process of augmentation. Since hesperidin methyl chalcone failed to induce augmentation, it was assumed that the gonadotropic-augmenting properties of the other compounds were associated with their antihyaluronidase activity and not with their antiproteolytic effects. The augmentation of FSH by antihyaluronidase compounds is attributed to a slow release of the gonadotropin from the site of injection by inhibition of endogenous hyaluronidase activity.

66. Sublingual Ergot Alkaloids (Hydergine) in the Treatment of Vasomotor Flushes.

M. James Whitelaw, M.D. and *Roberto F. Escamilla, M.D.*

Endocrine Clinic, University of California School of Medicine, San Francisco, Calif.

It is generally agreed that in patients with breast dyscrasias, a history of mammary or genital-tract carcinoma, or a familial history of cancer, estrogen therapy is contraindicated for treatment of the menopausal syndrome. In certain climacteric patients, receiving cyclic estrogen therapy, there is a return of symptoms during the period of withdrawal. Hydergine sublingual tablets, each containing 0.5 mg. of hydrogenated ergot alkaloids were tested solely for their ability to depress flushes in 26 women in this latter group. Hydergine has a central action, decreasing vascular tone, enhanced by peripheral vasodilation. It has an adrenosympathicolytic action at the peripheral effector organs.

Results: 2 failures, 8 cases with partial relief, and 16 cases with good results. Among the latter patients, 10 received placebos alternately, which resulted in failure in 6 cases, partial relief in 3 and good results in 1.

Three x-ray castrated women who were menstruating previous to therapy for breast carcinoma, had partial relief from therapy. Six women with breast dyscrasias were treated alternately with placebos; there were 4 failures and 2 cases of partial relief. With Hydergine there was significant relief in 4 cases, partial relief in 1, and failure in 1. No side reactions were noted.

The advantages of Hydergine, as well as its disadvantages, are discussed.

67. Female Hypogonadism Untreated Until the Fifth Decade of Life.

Robert F. Skeels, M.D., E. Kost Shelton, M.D. and *Henry H. Turner, M.D.*

The Shelton Clinic, Los Angeles, California and The University of Oklahoma School of Medicine, Oklahoma City, Okla.

Estrogen-withdrawal bleeding was induced for the first time in 3 hypogonadal women at ages 41, 50 and 63 years. Two were classic cases of Turner's syndrome with congenital anomalies and ovarian agenesis. The third was eunuchoid with low FSH. All

were emotionally immature, maladjusted, and depressed, and one was actually prepsychotic. Endocrine treatment was instituted at this late date in life with the hope that their mental outlook might be improved. At first, treatment was resisted because of painful pelvic congestion; as it was continued, this was interpreted as sexual excitation. Eventually morale and well-being were improved.

Symptoms referable to osteoporosis were alleviated in the 2 patients who continued treatment for thirty and thirty-three months, respectively. Sexual organs were capable of being developed even after many years of congenital ovarian deficiency. The eunuchoidal patient discontinued treatment after several months because of an acute psychotic episode which required electroshock. The hormonal therapy may or may not have contributed to this breakdown. The other 2 patients, previous chronic complainers, continue to take their hormones, feel well, and are reasonably happy. Therefore, age in itself is not necessarily a contraindication to the estrogen treatment of previously neglected hypogonadism.

68. The "Normalizing Steroids." Are We Missing the Boat?

John P. U. McLeod, M.D.

Marshville, N. C.

These observations are based on a 3-year study of the use of an oral androgen-estrogen combination in 650 patients. The clinical findings, although not substantiated by exhaustive laboratory studies, suggest that these steroids, possibly because of their "sex" connotations, have not been generally exploited to their full extent. This study tends to suggest that, besides their well known uses in menopausal and climacteric syndromes and in certain sex-associated malignancies or their less well known uses in bone or skin metabolism and in senile asthenia, these steroids actually may be used in practically every branch of medicine and in most of the infirmities that beset mankind. The data suggest that these steroids have a great "normalizing" effect on the pathologic secretions of the endogenous hormones, a potentiating effect on administered hormones (while reducing their side reactions), a soothing effect on the dysfunctions of the sympathetic-parasympathetic nervous system or the irritable cerebral cortex, and an almost euphoric effect on the psychiatric depressions. They raise the pain threshold of the supersensitive, or at least reduce the patient's awareness of pain or other disagreeable stimuli. Thus they reduce the deleterious effects of "stress" by reducing the "alarm" and the "alarm reactions," by increasing and prolonging the "stage of resistance," and by stabilizing the delicate balance of the psycho-hypothalamico-hypophyso-adrenocortico-gonadal axis. These observations suggest that the practice of medicine may soon consist of a close amalgamation of endocrinology with psychiatry, assisted by pharmacologic and dietary consultants, and that surgery will be relegated to a minor role of helping with an occasional failure-to-respond patient and caring for the inevitable accident victims.

69. Prevention by Chlorpromazine of the Nausea Caused by Estrogens and Estrogen-Like Compounds.

M. James Whitelaw, M.D.

San Francisco, Calif.

Fourteen male volunteers were given a 25-mg. uncoated stilbestrol tablet and also a

25-mg. Chlorpromazine capsule. No nausea was experienced by any patient. Six were retested without the anti-emetic six weeks later and 4 noted nausea of varying degree. Three volunteer females were given stilbestrol (1–5 mg. or 2–10 mg.), which produced definite nausea. This was prevented by adding Chlorpromazine and repeating the experiment. Seventy-four male and female patients, who were unable to tolerate stilbenes, hexanes, synthetic or natural estrogens because of nausea, were given Chlorpromazine in doses varying from 25–200 mg. daily. In only 1 patient was there a complete failure; all others were able to continue therapy. The only side effects noted were marked sedation in 2 cases, mild sedation in 16 cases; transient tachycardia in 1 case; and dryness of the mouth in 4 cases.

70. Interference With Metabolism of C^{14}-Labeled Estrone in Aminopterin-Treated Rats.

J. B. Trunnell, M.D. and *Wendell E. Bowman, Ph.D.* (by invitation), with the technical assistance of *Hazel E. Hildebrandt, B.S.*

The Section of Experimental Medicine, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Four Sprague Dawley rats, 2 males and 2 females (one of each being folic-acid "deficient"), received doses of 16-C^{14} estrone subcutaneously, and were placed immediately in individual all-glass metabolism cages. Urine, feces and exhaled CO_2 were collected separately for eighteen hours and the animals were then killed and dissected. After freeze-drying, the various (56 per animal) samples of tissue were extracted and counted in a flow counter. In a few instances the extracts were chromatographed and autoradiograms thereof exposed. In almost all instances in which a difference was observed, the tissues of the deficient animals contained more radioactive carbon than did those of the controls. Particularly noteworthy in this respect were liver, pancreas, hypophysis, ventral and dorsal prostate, seminal vesicle, coagulating gland, uterus, vagina, cartilage, salivary gland, spleen, stomach and urine. The thyroid glands of both females contained five times as much activity as did those of the males.

71. Aberrations of Estrone Metabolism in Rat Hepatoma.

Clara M. Szego, Ph.D. and *Dorothy C. Wolcott, B.A.*

Department of Zoology, University of California, Los Angeles, Calif.

The influence of surviving rat liver on the metabolism of estrone-16 C^{14} in an homologous serum medium has been reported from these laboratories (*Endocrinology* **52**: 669, 1953). An enzyme system which promotes the binding of estrone or its metabolites to the serum proteins under the conditions described occurs in normal liver, is augmented in hepatic tissue regenerating after surgical or dietary injury, and is virtually absent from hepatoma, either transplanted or induced by incorporation of DAB into the diet (*Fed. Proc.* **13**: 1954). Additional aberrations of estrone metabolism in the presence of surviving hepatoma have been detected. Recovery of estrone or its metabolites from the liver proteins per se, was significantly and consistently depressed. Essentially complete recovery of the added radioactivity was observed in the various fractions of the incubate analyzed in the case of the tumors. In the control experiments, there was evidence of degradation of the steroid, in part to relatively acidic substances, with some metabolites escaping detection entirely, by the methods used. The tumor tissues appeared defective in this regard. These experiments appear to constitute an advance toward the bio-

chemical characterization of neoplastic liver and aid in the elucidation of normal metabolic pathways for the estrogens.

72. Influence of Steroids on Uterine Protein Metabolism.

Sidney Roberts, Ph.D. and *Howard Hoffman, M.S.* (by invitation).

Department of Physiological Chemistry, School of Medicine, University of California Medical Center, Los Angeles, Calif.

Techniques have been developed which permit separation of the protein components present in extracts of single rat uteri, weighing 100 to 150 mg. or in the dissected endometrial and myometrial components of this tissue. Micro-extraction procedures at low temperatures and a suitable modification of the filter-paper electrophoresis technique were employed. The distribution of protein components was similar to that obtained by conventional electrophoresis of larger-scale uterine extracts. Incorporation of isotope into the separated proteins of endometrium and myometrium obtained from animals injected with labeled amino acids was estimated by direct measurement of the radioactivity present on the filter-paper strip. Twenty hours after intravenous administration of 30 μc. of phenylalanine-3C^{14} into castrated rats, little radioactivity was recovered in extracts of the whole uterus. Net incorporation of this amino acid into the uterine proteins was markedly enhanced in animals injected with estradiol twenty hours earlier. Stimulation of incorporation into one of the four protein components separated by paper electrophoresis was particularly striking. When hydrocortisone was administered with estradiol, incorporation of isotope into all uterine proteins was depressed. This depression was particularly prominent for the same component which responded selectively to injection of estrogen alone.

73. Studies of Vaginal Keratinization With the Aid of Radiocystine.

Howard A. Bern, Ph.D., Donald R. Harkness (by invitation) and *Sidney M. Blair* (by invitation).

Zoology Department and its Cancer Research Genetics Laboratory, University of California, Berkeley, Calif.

Radioautographic and tracer methods have been used to study the uptake of S^{35}-labelled cystine by the female mouse genital tract in response to hormones. Both vagina and uterus from estrogen-treated and androgen-treated castrated mice show a similar uptake of radiocystine administered eight hours prior to sacrifice, which is greater than in the sesame oil-treated castrated controls. This increment corresponds to the hyperplasia and hypertrophy induced by the steroids. Radioautographic studies showed that a radioactive keratin layer is obtained in the estrogen-treated vagina when radiocystine is administered forty-eight hours prior to sacrifice of the animal. Radioautographs of the estrogen-treated keratinizing vagina are more positive twenty-four and forty-eight hours after radiocystine injections than those of the androgen-treated mucifying vagina. This may be ascribed to incorporation of the cystine into keratin and consequent lack of turnover. To date we have no evidence for a specific uptake of cystine by the vaginal epithelium undergoing cornification, beyond that expected as a result of growth stimulation, despite the recognized importance of disulfide bonds in keratin formation. A possible difference in the incorporation of cystine in vaginal and epidermal keratinization on the one hand, and in hair and lingual papilla keratinization on the other hand, is discussed.

74. Changes in the Uterine Cervix and Vulva of the Gilt Following Treatment with Relaxin.

M. X. Zarrow, Ph.D., D. Sikes, D.V.M. (by invitation) and *G. M. Neher, Ph.D.* (by invitation).

Dept. of Biological Science and Dept. of Veterinary Science, Purdue University, Lafayette, Ind.

Castrated gilts were treated with stilbestrol and a combination of stilbestrol and relaxin. The diameter of the cervical canal was measured by the passage of aluminum rods of different diameters and all animals were injected with Evans blue prior to death. The cervical canal of all animals following castration permitted the passage of a rod with a diameter of $\frac{3}{8}$ inch. No further change was observed in the control animal but the gilt treated with 5 mg. of stilbestrol daily for ten days permitted passage of a $\frac{1}{2}$-inch rod within forty-eight hours—a condition which remained unchanged during the next eight days of treatment. Gilt No. 1 was injected with stilbestrol for seven days, followed by 5,000 GPU of relaxin 3 times daily for four days. This treatment caused a dilation of the cervical canal permitting the passage of rods up to 1 inch in diameter. A second gilt, pretreated with stilbestrol and then with 9,000 GPU of relaxin 3 times daily for four days, also showed a marked dilation of the cervical canal. Histochemical examination revealed an increased permeability and a depolymerization of the ground substance in the cervix of the relaxin-treated animals. This was confirmed by studies indicating an increased water content in the cervix, vulva and uterus of the treated gilts. The vulva showed extensive edema during relaxin treatment and appeared to approximate the condition seen at parturition.

75. Study of Collagen Fibers in Normal and Pathologic Human Testes.

F. A. de la Balze, M.D., R. E. Mancini, M.D. (by invitation), *O. Vilar, M.D.* (by invitation), *J. Irazu, M.D.* (by invitation) and *J. Nolazco, M.D.* (by invitation).

III Catedra de Clinica Medica de la Facultad de Medicina, Buenos Aires, Argentina.

Morphology and distribution of reticular and collagen fibers were studied by biopsies of 38 normal and 75 pathologic testes.

Formol fixation and Del Rio Ortega's silver impregnation were used for microscopic demonstration of fibers.

Normal testes showed:

Adults. Reticular fibers gathered together in the basal membrane, forming a network of thick fibers in the tunica propria. They were scarce, thin and irregularly distributed in the intertubular connective tissue where they tended to form a net around each Leydig cell, particularly when these were groups together. The albuginea was almost completely formed by collagen fibers.

Newborn. Reticular fibers formed a thin net in the basal membrane but were abundant in the intertubular connective tissue where they surrounded interstitial cells. They were present also in the albuginea and interlobar septa.

Prepuberal boys. Reticular fibers gathered together in the basal membrane; they decreased in the intertubular connective tissue.

Puberal boys. Testes were in various stages of development towards adulthood. With maturity, reticular fibers gathered together in the basal membrane and formed a well

organized, loose net of thick reticular fibers in the tunica propria. In the intertubular connective tissue, when Leydig cells grouped, reticular fibers formed a net around each cell thus resembling the adult picture.

The changes observed in endocrine and nonendocrine abnormalities are presented.

76. Etiologic Factors in Spermatogenic Arrest.

Warren O. Nelson, Ph.D.

University of Iowa College of Medicine, Iowa City, Iowa.

Spermatogenic arrest represents one of the most frequently seen testicular defects in men with azoospermia (61 of 238 cases). It is marked in all but very exceptional cases by the arrest of spermatogenesis at the primary spermatocyte stage. Rather than continuing the maturation process by undergoing a meiotic division, the germ cells show nuclear pycnosis or fragmentation in a fashion suggesting that the mechanisms responsible for meiosis are effectively blocked. This defect is seen 1) when the body temperature is significantly elevated (*e.g.*, pneumonia, chicken pox), 2) in cases of scrotal insulation, 3) when the individual is subjected to high occupational temperatures, 4) when the testes are high in the scrotum, 5) in cases of gonadotropin deficiency related to eunuchoidism, tumors of the hypophysis, after hypophysectomy, and after x-irradiation of the hypophysis, and 6) following administration of certain drugs. In other instances no etiologic factor can be determined. It is possible to correct the defect in those cases in which the causative factor can be determined and eliminated. It is noteworthy that spermatogenic arrest produced in rats by nitrofuran compounds apparently is related to an interference with enzymatic mechanisms involved in the metabolism of carbohydrate by testicular tissue. It is uncertain whether other cases of arrest have a similar relationship.

77. Influence of 19-Nortestosterone Cyclopentylpropionate on the Urinary Nitrogen of the Castrated Male Rat.

Robert O. Stafford, Ph.D., Barbara J. Bowman, B.A. (by invitation) and *Kenneth J. Olson, Ph.D.*

The Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

19-Nortestosterone cyclopentylpropionate (NTCP) has been found to have one fifth the activity of testosterone propionate in stimulating the seminal vesicle of the castrated rat, and to have nearly twice the activity of testosterone propionate in causing growth of the levator ani muscle. The present study describes semiquantitative measures of the ability of this compound to cause retention of nitrogen in adult castrated male rats force-fed a constant intake of medium carbohydrate diet.

Comparison with testosterone propionate (TP) in thirty-day courses of injection revealed that 0.5 mg. per day of NTCP produced a greater nitrogen response than did 0.25 mg. of TP, but a lesser response than 1.0 mg. of TP; 2.0 mg. of NTCP produced the same response as 0.5 mg. of NTCP.

NTCP in a daily dose of 0.25 mg. caused greater nitrogen retention than did androstanolone (17β-hydroxy androstane-3-one) or methylandrostenediol (17α-methyl Δ^5-androstene-3β,17β-diol) in daily doses of 2.0 mg.

A single injection of 2 mg. of NTCP caused significant retention of nitrogen lasting several days, whereas a similar injection of 19-nortestosterone did not produce significant retention.

78. Testosterone Isobutyrate and Testosterone Phenylacetate: Two Long-Acting Androgens for Clinical Use.

Joseph M. Looney, M.D.

Veterans Administration Regional Office, Boston, Mass.

Aqueous suspensions of testosterone isobutyrate, and of testosterone phenylacetate containing 1 per cent procaine and 0.5 per cent chlorobutanol can produce androgenic action lasting three to four weeks. These preparations have been used in 3 cases of panhypopituitarism, 3 cases of hypogonadism and in 1 case of acromegaly. Two of the panhypopituitary failures resulted from x-ray therapy for adenoma. In 1 of these, 300 mg. of testosterone isobutyrate every two weeks replaced 800 mg. of cyclopentyltestosterone. In the other case, 200 mg. of testosterone isobutyrate every two weeks was substituted for 25 mg. of testosterone propionate three times a week. The third case required nailing of a hip for a slipped epiphysis; during androgenic and thyroid therapy, fusion of the epiphyses occurred. A single injection of 400 mg of testosterone isobutyrate once a month was as effective as 25 mg. of testosterone propionate three times a week. Two cases of infantilism (one in a 27-year-old man with untreated cryptorchism and the other in a 25-year-old man following childhood mumps) responded well to 400 mg. of testosterone phenylacetate once monthly. In the third case of hypogonadism (a 35-year-old married veteran who had lost both testes from gunshot wound, the patient has been able to have satisfactory marital relations during treatment with a monthly injection of 400 mg. of testosterone phenylacetate, replacing 25 mg. of testosterone propionate three times a week. The output of 17-ketosteroids was markedly increased. The final case is that of a 33-year-old acromegalic who had had x-ray therapy and complained of severe headaches. He received considerable relief from 400 mg. of either preparation once a month. Half of the patients complained of soreness lasting for about five to six days, with the isobutyrate. This either did not occur with the phenylacetate preparation or, at most, lasted for only a few hours.

THYROID

79. Goiter in Uruguay and Chile.

Isidor Greenwald, Ph.D.

New York University College of Medicine, New York, N. Y.

In a recent paper (*Ohio State M. J.* **49:** 313, 1953), Kimball wrote: "Endemic goiter, with its sequelae, is reported in every state of South America, with the exception of Chile and Uruguay, and this is due to their geographical limitations. In no country do we find many cases of endemic goiter along the seacoast." These statements are erroneous. There were at least fifteen papers dealing with goiter, hyperthyroidism and hypothyroidism published in Uruguay between 1928 and 1951. The three places in which it was most prevalent are *at, or near, the coast.* In Chile, goiter was present before 1821. It became increasingly prevalent. Its presence is mentioned by Hirsch. In 1951, Feferholtz and Ortiz (*Rev. méd. de Chile,* **79:** 22) wrote of Boco, a village adjoining Quillota, a town about twenty-five miles from the sea and less than 1,000 feet above it, that goiter was so common as to give one the impression that one had been transferred to a village in Switzerland ("peso sobre uno la impression de hallarse traslado a algun pueblo de Suiza").

80. Four-Year Study of the Treatment of Hyperthyroidism with Methimazole.

Thomas H. McGavack, M.D., Jacqueline Chevalley, M.D., Sydney Pearson, Ph.D. and Samuel Kenigsberg, M.D.

The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

Methimazole (1-methyl-2-mercaptoimidazole) has been administered to 184 patients with hyperthyroidism in initial daily doses ranging from 15 to 60 milligrams and in maintenance doses of from 2.5 to 30 milligrams for periods of time varying from fourteen days to four years. If the dosage was adequate, improvement was observed in three fourths of the patients within the first week of therapy. Control of the thyrotoxic state required from two to twenty-six weeks, with 85 per cent of the subjects euthyroid within the first five weeks. If patients are excluded in whom the initial daily doses were less than 20 mg., then 95 per cent of the remaining subjects became nontoxic within five weeks. Of 90 patients in whom the size of the thyroid was serially followed during treatment, 12 showed an increase, 45 a decrease and 33 no appreciable change in the size of the gland. In 37 patients, 35 with a nodular and 2 with a diffusely hyperplastic goiter, methimazole was used solely as a preoperative measure. Of the remaining 147 cases, it has been possible to obtain a satisfactory follow-up in 96. Of these 96 patients, 41 were still receiving methimazole when the series was closed; 42 had completely recovered, of whom 8 had a recurrence of the thyrotoxic state; 7 had been operated on; and 6 had been given radioactive iodine. Toxic manifestations appeared in 8 of the 184 patients. The highest percentage of "cures" was observed in patients below 35 years of age. In middled aged persons, *i.e.*, between 35 and 54 years of age, the disease was brought under control less rapidly than in either the younger or the older individuals.

81. Granulocytopenic Reactions in Man to Antithyroid Compounds.

Thomas H. McGavack, M.D., and *Jacqueline Chevalley, M.D.*

The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

For all practical purposes, granulocytopenia, or the more severe agranulocytosis, is the one bone marrow reaction with which the physician is concerned. In man, derivatives of aniline, thiourea, thiazole, thiobarbituric acid, thiouracil and thioimidazole have been employed for their possible influence upon thyrotoxicosis. Aminothiazole, thiourea, thiobarbital and thiouracil are too toxic for routine use. In effective dosages, methylthiouracil, propylthiouracil and methimazole are about equally toxic. The incidence of granulocytopenia is directly proportional to the dosage of antithyroid compound and the duration of its administration. Reactions of the type under consideration have rarely been reported before the tenth day and are more common beyond the third week of administration. This fact, and the high concentrations of antithyroid compounds found in bone marrow, indicate a direct toxic action upon the medullary cavity. A review of cases of granulocytopenia produced by the various compounds indicates that the reactions occurring following methimazole are less rapidly progressive than those seen with thiouracil, methylthiouracil or propylthiouracil.

82. Effect of Insulin on the Thyroid Gland and Its Influence on Heat Regulation.

Boris Catz, M.D., M.S., Ernest Geiger, M.D., Ph.D. (by invitation), *Heriberto Thomas, M.S.* (by invitation) and *Ihsan El-Rawi, Ph.D.* (by invitation).

Dept. of Medicine and Pharmacology, University of Southern California, Los Angeles, Calif.

In earlier experiments one of the authors (E. G.) indicated the role of insulin in heat regulation. This was further investigated as follows:

In one group of experiments the effect of insulin on the thyroid gland was studied in Wistar strain male rats (weighing 90 to 125 Gm.) exposed to cold (2°-3° C.) and to room temperature (24°–26° C.) As shown in the table the animals receiving regular insulin (Iletin, Lilly), 0.5 unit b.i.d. subcutaneously for eight days, gained significantly more weight than the controls and this difference was more pronounced in the cold-exposed rats.

The thyroid gland structure as measured by microhistometry revealed the following changes:

1. At normal room temperature the thyroids of the insulin-treated rats showed a definite hyperplasia—mean acinar cell height (M.A.C.H.) 14.1 micra (controls—11.2 micra).

2. In the cold room, however, the thyroids of the insulin-treated rats were less hyperplastic (12.4 micra) than those of the controls (14.0 micra).

The histologic changes and the increase in weight that occurred in the insulin-treated animals suggest a close interrelationship between insulin and the thyroid gland in their effect on heat regulation.

In another group of experiments we studied the effect of insulin on the survival of normal rats and rats receiving 0.1 per cent thiouracil in the drinking water. Fifty per cent of the thiouracil-treated rats died after injection of 1 unit of insulin, and none survived 8 units, whereas 75 per cent of the normal rats survived the dose of 10 units. Furthermore, the thyroid gland of the thiouracil-insulin treated animals showed less hyperplasia microhistometrically (14.7 micra) than the controls treated with thiouracil alone (17.0 micra). These survival results are similar to those obtained by Ducheneau (1924) in thyroidectomized rabbits.

Determination of the liver glycogen and histologic study of the adrenals are in progress.

TABLE

Condition	No. of rats	Av. wt. gain (Gm.)	Thyroid M.A.C.H. (micra)
Saline 0.1 cc., 8 days, 24°–26° C.	9	37	11.2
Insulin 0.5 U b.i.d., 8 days, 24°–26° C.	5	55	14.1
Saline 0.1 cc., 8 days, 2°–3° C.	10	18	14.0
Insulin, 0.5 U b.i.d., 8 days, 2°–3° C.	6	42	12.4

83. Effect of Hyperthyroidism and Hypothyroidism on Young Fat-Deficient Male Rats.

Theodore C. Panos, M.D. and *John C. Finerty, Ph.D.*

University of Texas Medical Branch, Galveston, Texas.

Male Holtzman weanling rats were grouped as follows: 42 were started on a synthetic fat-free diet and 32 were fed Purina Laboratory Chow. Of the former, 12 were thyroid-

ectomized and 20 were fed 0.5 per cent desiccated thyroid power (Armour). Of the chow-fed group, 12 were thyroidectomized and 10 were fed thyroid powder. During the 16-day experimental period, 8 deaths occurred in the fat-free group receiving thyroid, whereas there were no deaths in other groups. Weight gain, food intake and general condition were observed. At the time of sacrifice, the endocrine glands, liver, heart, kidneys and blood were taken for study. The fat-free diet alone caused retarded growth and increased the relative weight of the brain, kidneys, liver and heart. Hyperthyroidism markedly increased the relative weight of the brain and both relative and absolute weight of the kidneys, liver and heart; these changes were accentuated in the fat-free group. Hypothyroidism reduced the relative weight of the kidneys, liver and heart, and effectively prevented the enlargement of these organs regularly observed in fat deficiency. These data suggest that thyroid function is intimately involved in the metabolic effects of fat deficiency.

84. Metabolism of I^{131}-Labeled Thyroxine by an Isolated Perfused Rabbit Liver.

David V. Becker, M.D., Leon A. Woods, Jr., M.S., Kenneth G. DuBois, M.A. and *John F. Prudden, M.D.* (introduced by Vincent P. Hollander, M.D.).

Surgical Research Unit, Brooke Army Medical Center, Ft. Sam Houston, Texas.

A considerable body of evidence exists implicating the liver in the metabolism of thyroxine. The hepatic metabolism of I^{131}-labeled thyroxine was studied with an isolated rabbit liver in a simplified perfusion apparatus. Total and protein-bound radioactivity of the perfusate were determined and selected samples were chromatographed, utilizing a one-dimensional butanol-dioxane-ammonia paper system. Bile obtained at the end of the study was examined in a similar fashion, as were extracts of the liver tissue.

The viability of the preparation was confirmed by histologic examination and by chemical data. Both total protein and albumin of the perfusate were maintained at a constant level. Urea nitrogen increased steadily through the duration of the study, indicating the active synthesis of urea. The total radioactivity in the perfusate decreased in an exponential fashion with a half-life on the order of 20 hours. The protein-bound activity declined in a two-component fashion, with half-lives on the order of 2–4 and 35–40 hours, respectively. Chromatographic evidence of the integrity of the thyroxine in the perfusate was obtained, as well as evidence of its presence in the bile. Triiodothyronine could not be found in the perfusate. Further work is planned.

85. Effect of Thyroxine and Triiodothyronine on Electroshock Seizure Threshold in Intact and Adrenalectomized Rats.

P. S. Timiras, M.D., S. Agarwal, M.D. (by invitation) and *D. M. Woodbury, Ph.D.*

Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Mich.

We have shown previously (Woodbury *et al.; J. Pharmacol. & Exper. Therap.* **106**: 331, 1952) that thyroxine increases brain excitability in rats, as measured by electroshock seizure threshold. The present study was undertaken to investigate: a) the effect of triiodothyronine on brain excitability as compared with that of thyroxine, and b) the role of the adrenal cortex on the influence of thyroxine and triiodothyronine on brain

excitability. In intact rats, triiodothyronine, on a weight basis, is five times more potent than thyroxine in its ability to increase brain excitability. Both thyroxine and triiodothyronine cause an increase in adrenal weight and a decrease in thymus weight; this constitutes evidence of stimulation of secretion of adrenocortical hormones. Adrenalectomy markedly diminishes the effect of thyroxine on brain excitability. Since it has been observed in this laboratory that both cortisone and hydrocortisone increase brain excitability (Woodbury: *J. Pharmacol. & Exper. Therap.* **105:** 27, 1952), the lower effectiveness of thyroxine in the absence of the adrenal suggests that stimulation of the adrenal cortex by thyroxine is responsible, at least in part, for the increase in brain excitability produced by thyroxine. Adrenalectomy also decreases the augmenting effect of triiodothyronine upon brain excitability, but to a much smaller extent than that of thyroxine. Since alterations in brain excitability have been correlated with concomitant changes in brain and plasma electrolytes (Woodbury: *Recent Progr. Hormone Res.*, in press), Na, K and Cl concentrations in plasma, brain and muscle were determined in the present experiments and correlated with the corresponding changes in brain excitability; the results are presented.

86. Effect of Certain Metabolic Alterations on the Subcellular Distribution of I^{131}-Labeled Thyroxine and Triiodothyronine.

Paul VanArsdel, Jr., M.D. (by invitation), *Ruth Miller* (by invitation), *John R. Hogness, M.D.* and *Robert H. Williams, M.D.*

Dept. of Medicine, University of Washington School of Medicine, Seattle, Wash.

L-thyroxine and 3,5,3′*l*-triiodothyronine, labelled with I^{131}, were administered intravenously to normal Sprague-Dawley rats. At short intervals following injection the animals were sacrificed, and nuclear, mitochondrial, microsomal, and soluble fractions of liver, muscle and kidney were obtained by differential ultracentrifugation as outlined by Lee and Williams. Concentration of radioactivity in these compartments was compared with that in rats in which the metabolic state was altered by pretreatment with excess nonlabeled thyroxine, or by subcutaneous administration of epinephrine. In normal rats, the two hormones showed the greatest concentration in the microsomal fraction of liver and muscle, and in the soluble fraction of kidney. Thyroxine showed a preferential concentration in the microsomes in all three tissues, whereas triiodothyronine was found in greater concentration than thyroxine in the soluble fraction, the greatest difference being in kidney. Epinephrine showed its greatest effect on muscle, in which the concentration of both hormones in the microsomal fraction was reduced. In addition, a significant shift of triiodothyronine from the mitochondria to the soluble fraction occurred. In kidney, epinephrine produced a higher concentration of both hormones in all fractions, with the soluble fraction showing the greatest increase. In liver, the main effect of epinephrine was to increase the concentration of triiodothyronine in the soluble fraction and mitochondria; preliminary work shows that the concentration of thyroxine in these compartments is reduced. The presence of excess thyroxine in the body reduced distribution of radioactivity significantly in the soluble fraction and microsomes of all three tissues, with variable effects on distribution in the other compartments. Interpretation of the foregoing data, and data obtained from rats in which the metabolic state was altered, are discussed in relation to what is known about the effect of thyroxine and triiodothyronine on enzyme systems and the relative activity of these systems in different subcellular compartments.

ADRENALS

87. Changes in Urinary 17-Hydroxycorticosteroid Excretion During Stressful Life Experiences in Man.

Basil S. Hetzel, M.D., William W. Schottstaedt, M.D., William J. Grace, M.D. and *Harold G. Wolff, M.D.* (introduced by Willard O. Thompson, M.D.).

The New York Hospital, New York 21, N. Y.

Determinations of urinary 17-hydroxycorticosteroid excretion have been carried out, using a modification of the method of Nelson and Samuels. Each determination was made in duplicate and expressed as gamma per minute, the standard deviation of the mean being 0.64 gamma/minute in the range of values found. From four to twelve determinations were made in each of 10 healthy subjects over periods of one to eight hours. Under conditions of relative tranquillity the values were found to be in the range 1.0–4.7 gamma/minute; in certain stressful situations, provoking feelings of apprehension, anger or excitement, values from 5.6–8.3 gamma/minute were observed. These elevated levels were usually found to be associated with diuresis of water, nitrogen, sodium and potassium, though such diuresis was not always accompanied by an increase in 17-hydroxycorticosteroids. A series of observations throughout the day in 3 of these subjects showed a minimal variation during sleep and a maximal variation during the waking hours, in association with reaction to various stimuli.

88. Radiographic Visualization of the Adrenal Glands.

Eugene F. Poutasse, M.D.

Cleveland Clinic, Cleveland, Ohio.

Representative material covering all phases of radiographic visualization of the adrenals has been drawn from a group of approximately 80 cases, and the diagnostic value of such a test is assessed. Special technics are often required to visualize the adrenals, because of their location and tissue translucency, corresponding to that of the surrounding fat. A large adrenal tumor usually will cause renal displacement, as shown by flat film or intravenous urography. Suprarenal calcifications may be found in the case of primitive medullary tumors. Translumbar aortography has been of no practical value in showing small adrenal tumors. Laminography may be of value in locating small adrenal tumors even in an aberrant location, but interpretation of films is difficult. Presacral gas insufflation is the best method yet devised. It produces sufficient contrast to show both adrenals simultaneously and adequately.

89. Interrelationships of Liver Glycogen Deposition, Corticosteroids and Ascorbic Acid in Scorbutic Guinea Pigs.

A. S. Mulay, Ph.D. (by invitation), *L. D. Saslaw, B.S.* (by invitation) and *E. M. Nadel, M.D.*

National Institutes of Health, Bethesda, Md.

Hydrocortisone and 6,beta-hydroxyhydrocortisone have been crystallized from urinary extracts from severely scorbutic guinea pigs. Their identification by infrared spectroscopy (Burstein, Dorfman and Nadel: *Fed. Proc.* **13**: in press, 1954) extend and support observations based on ultraviolet spectroscopy and chemico-biologic assay (Nadel and Schneider: *Endocrinology* **51**: 5, 1952). The paradox of decreased amounts of glyco-

gen in the liver of scorbutic animals (Murray and Morgan: *J. Biol. Chem.* **163**: 401, 1946; McKee, Cobbey and Geiman: *Endocrinology* **45**: 21, 1949) despite higher than normal concentrations of hydrocortisone in the urine and blood (Nadel, Burstein and Dorfman: unpublished) was studied with the use of compounds E and F, glucose, alanine, fructose and ascorbic acid, given alone and in various combinations to normal and fasted controls and to scorbutic animals. Ascorbic acid given alone on the day of assay was not glycogenic. The high urinary corticoid excretion and low liver glycogen content of scorbutic animals were both reversed towards normal by the administration of ascorbic acid the day prior to assay. Glucocorticosteroids and known glycogen precursors were relatively ineffective in the deposition of liver glycogen in scorbutic animals, in contrast to their effectiveness in control animals. Administration of ascorbic acid together with either compound E or glucose or both, was associated with enhanced glycogen deposition in scorbutic animals.

90. A Simplified Test of Adrenocortical Function, Utilizing Corticotropin Gel.

R. Orr, M.D. (by invitation), *V. Di Raimondo, M.D.* (by invitation), *A. Rinfret, Ph.D.* (by invitation), *D. Island, B.S.* (by invitation) and *P. H. Forsham, M.D.*

The Metabolic Unit, University of California School of Medicine, San Francisco, Calif.

In the quantitative measurement of adrenocortical function, as reflected by changes in urinary corticoid excretion following ACTH stimulation, the stimulus must be constant in magnitude and duration. This constancy has previously been achieved by the intravenous infusion of a standard dose of ACTH over a fixed period.

A modification of this method, utilizing ACTH gel as a stimulus, has been developed. By selecting a dose of commercial, high-potency ACTH gel in excess of that necessary for maximal stimulation, *viz.*, 80 units, and limiting the period of urine collection following the administration of the ACTH gel to eight hours, a stimulus of constant magnitude and duration is provided. The amount of total 17-hydroxycorticoids contained in the eight-hour post-stimulation urine specimen, when compared to basal urinary output, is a quantitative measure of adrenocortical responsiveness to stimulation. Adrenal function, as estimated in this manner, compares closely with the values obtained using the eight-hour intravenous ACTH test, with the obvious advantage that it may be performed on ambulatory as well as immobilized subjects. These findings are illustrated.

91. Adrenocortical Response to Low-Dose ACTH Stimulation in Cushing's Syndrome, Adrenogenital Syndrome and Normal Subjects.

Richmond W. Smith, Jr., M.D., Raymond C. Mellinger, M.D. (by invitation) and *Anne A. Patti, M.S.* (by invitation).

The Division of Endocrinology, Henry Ford Hospital, Detroit 2, Mich.

Urinary 17-hydroxycorticoids in patients with Cushing's syndrome usually exceed normal values and when overlapping occurs, diagnoses are made from other indices. In this study, the adrenal response to a low-dosage of Wilson Corticotropin-gel (1.7 or 3.5 U.S.P. units twice daily for two days) has been measured in 5 patients with Cushing's syndrome, 2 with adrenogenital syndrome, 5 with borderline features, and 12 normal subjects. Circulating eosinophils, and urinary creatinine, 17-hydroxycorticoids and 17-ketosteroids were determined. Indices evaluated were: 1) the percentage reduction in eosinophils; 2) the basal 17-hydroxycorticoid excretion, mg./day; 3) the basal 17-hy-

droxycorticoid excretion, mg./1000 mg. of creatinine; 4) the percentage increase of 17-hydroxycorticoid excretion (second day of ACTH); 5) the total increase of 17-hydroxycorticoid excretion, mg./1000 mg. creatinine/unit of ACTH; 6) the product of 3) and 5); and 7) the day-night 17-hydroxycorticoid excretion ratio. Comparing Cushing's syndrome with the normal state, indices 1) and 4) did not distinguish; index 2) values overlapped; indices 3) and 5) barely distinguished; and indices 6) and 7) clearly distinguished. In the adrenogenital syndrome, index 2) was normal, but indices 1) and 3) through 6) were extremely low. Borderline patients had a normal to high index 2), and normal to low indices for 3) through 6). Similar indices were made for 17-ketosteroid excretion. Hyperfunctioning cortical tumors (not studied) might be distinguished from hyperplasia by such analysis.

92. An Improved and Simplified Assay of Corticotropin, Utilizing Normal Male Subjects.

V. Di Raimondo, M.D. (by invitation), *A. Rinfret, Ph.D.* (by invitation), *D. Island, B.S.* (by invitation) and *P. H. Forsham, M.D.*

The Metabolic Unit, University of California School of Medicine, San Francisco, Calif.

Steroidogenesis is utilized as a measure of adrenocortical activation, as has previously been proposed by Liddle. Normal young males are given varying doses of corticotropin in 16 per cent gelatin intramuscularly at weekly intervals and, from an empty bladder, a urine sample is collected for exactly eight hours beginning immediately after injection. 17-Hydroxycorticoids are estimated, using a modification of the method of Reddy. To each subject a dose of 80 U.S.P. or clinical units is given in order to obtain a maximal 17-hydroxycorticoid output. Results obtained with lower doses are expressed as a percentage of the maximal. A linear relationship may be established between the percentage of maximum 17-hydroxycorticoid output and the logarithm of the dose of ACTH between 2.5 and 10 U.S.P. units, with a regression formula of $y = 11.7 + 79.9$ and an index of precision better than 0.2. Purified corticotropin of unknown potency may be assayed by using 5 subjects at two dose levels situated on the linear portion of the assay curve. Applications are illustrated.

93. Activity of Crude and Purified ACTH in the Presence of Enzyme Inhibitor.

Herman Cohen, Ph.D., William Kleinberg, Ph.D. (by invitation), *Henry H. Freedman, Ph.D.* (by invitation) and *Gustav Martin, D.Sc.* (by invitation).

Princeton Laboratories, Inc., Princeton, N. J., and National Drug Co., Philadelphia, Pa.

The purification of corticotropin by means of oxycellulose has resulted in preparations which are not as amenable to subcutaneous or intramuscular inactivation as are the crude starting materials from which the purer corticotropin is derived. This phenomenon has been ascribed to the removal of proteinases during the purification process (Sayers, G.: *Recent Progr. Hormone Res.* **7**: 52, 1952).

Using the anti-enzyme compound, phosphorylated hesperidin, and the subcutaneous assay method of Thompson and Fisher (*Endocrinology* **52**: 496) we have attempted to evaluate the foregoing concept. As may be noted from the following table, crude corticotropin, when administered subcutaneously in aqueous solution to hypophysectomized rats, is much less potent than oxycellulose-purified ACTH similarly administered. How-

ever, when injected in a 2.5 per cent solution of phosphorylated hesperidin, no difference between the two types of preparations is noted.

These findings are attributed 1) to the effectiveness of the phosphorylated hesperidin in inhibiting the tissue and exogenous proteinases acting upon the crude ACTH, and 2) to the anti-hyaluronidase action of the hesperidin, which decreases the tissue surface to which the ACTH is exposed, and delays its absorption into the blood stream.

Type of ACTH	Vehicle	No. of animals	Dose (U.S.P. units)	Ascorbic acid (mg./100 Gm. gland)
Purified	H_2O	5	0.20	312±17
Purified	"	5	0.067	430±15
Crude	"	5	0.35	370±12
Crude	"	5	0.117	408±30
Controls	"	5	0	470±15
Purified	2.5% H.ph*	9	0.20	253±13
Purified	"	9	0.067	322±15
Crude	"	9	0.20	266±19
Crude	"	9	0.067	323±11
Controls	"	4	0	441±18

* Phosphorylated hesperidin.

94. A Potent Steroidogenic Anterior Pituitary Fraction Which is Poor in Adrenal Ascorbic-Acid Depleting Activity.

Grant W. Liddle, M.D., Arthur P. Rinfret, Ph.D. (by invitation), *Donald Island, B.S.* (by invitation), *Don H. Nelson, M.D., Bernard Wexler, Ph.D.* (by invitation), *David Hume, M.D.* (by invitation) and *Peter H. Forsham, M.D.*

The National Institutes of Health and Naval Medical Research Institute, Bethesda, Md.; the Baxter Laboratories, Morton Grove, Ill.; and the Metabolic Unit, University of California School of Medicine, San Francisco, Calif.

Pituitary adrenocorticotropin (ACTH) induces (a) secretion of adrenal steroids, and (b) depletion of adrenal ascorbic acid. The former is of clinical importance; the latter is the basis for the official methods of assaying ACTH. In general, the two activities are correlated.

We have found, however, that a special fraction of hog anterior pituitary extract, "PX-la," shows a marked discrepancy between these two functions.

Assays by the Sayers method showed PX-la to be very poor in ascorbic-acid depleting activity (1.0 mg. of PX-la was equivalent to 0.04 mg. of U.S.P. standard ACTH), whereas assays in human subjects revealed PX-la to be unexpectedly potent in steroidogenic activity (1.0 mg. of PX-la was equivalent to 0.75 mg. of U.S.P. standard ACTH). All preparations were administered intravenously. Analysis of variance of respective assays showed the discrepancies between the ascorbic acid and steroidogenic functions to be significant at p 0.001.

The steroidogenic effectiveness of PX-la in a patient with panhypopituitarism and in hypophysectomized dogs indicated that PX-la acts on the adrenal cortex directly and not via the pituitary.

Steroidogenic potency was measured quantitatively in terms of (a) blood 17-hydroxycorticosteroids, (b) urinary 17-hydroxycorticoids, (c) urinary 17-ketosteroids, (d) circulating eosinophils, (e) potassium and sodium excretion, (f) nitrogen balance, (g) hyperglycemia, and (h) therapeutic responses in "collagen" diseases.

95. Congenital Adrenal Hyperplasia: Adolescent Development, Ovulation, Menstruation and Pregnancy Under the Influence of Cortisone.

Elizabeth Knapp Smith, Ph.D. and *R. R. de Alvarez, M.D.* (introduced by J. T. Bradbury, Sc.D.)

Dept. of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle, Wash.

Two young women, aged 18 and 34 years, with the adrenogenital syndrome resulting from congenital adrenal hyperplasia, have been treated with cortisone for twelve and eighteen months, respectively. Clinical progress was checked bi-weekly, including blood pressure, blood sugar level and the number of circulating eosinophils. Basal body temperature and vaginal smears were obtained daily. Urinary excretions of 17-ketosteroids, total estrogen, pregnanediol and gonadotropins were determined weekly.

For the younger girl, daily cortisone dosages of 100 mg. orally or 50 mg. intramuscularly were required to suppress androgen secretion sufficiently to permit feminization. Daily output of 17-ketosteroids decreased from pretreatment levels of 40–50 mg. to approximately 10 mg., at which level there occurred significant breast development, decreased hirsutism, vaginal cornification, and establishment of fairly normal menstrual cycles, confirmed by cyclic changes in urinary estrogen excretion.

In the older, married woman, oral cortisone in daily doses of 37.5–50 mg. decreased the control 17-ketosteroid values from 50–60 mg. to approximately 5 mg. daily. Ovarian activity was established promptly, and the patient became pregnant. During pregnancy, chorionic gonadotropin, estrogen and pregnanediol excretion values were essentially normal. Pregnancy terminated by spontaneous abortion at four months. Since then the patient has menstruated regularly, and basal body temperature, vaginal cytology and pregnanediol excretion indicate the occurrence of ovulation.

96. Studies of Steroid-Forming Neoplasms.

Julia Lobotsky, M.A. (by invitation), *Barbara P. Loewy, B.A.* (by invitation), *Charles W. Lloyd, M.D.* and *Walter F. Rogers, Jr., M.D.*

State University of New York, College of Medicine, Syracuse, N. Y.

A 39-year-old woman had amenorrhea, virilization, massive edema, hypertension and decreased glucose tolerance. 17-Ketosteroid excretion varied from 176 to 338 mg./twenty-four hours and 17-OH-corticosteroids from 32 to 212 mg./twenty-four hours. Autopsy showed bilateral adrenocortical carcinomata with pulmonary and hepatic metastases. An α-ketol, with an Rf on paper chromatography and sulfuric-acid chromogen spectrum identical with hydrocortisone, was found in the urine, the primary carcinoma and the metastases. Several unidentified spots were found on chromatography: one with an Rf approaching cortisone was in the urine, tumor and hepatic metastases. This had more salt-retaining ability than cortisone but less than desoxycorticosterone, and sulfuric-acid chromogen maxima at 282, 400–410 mμ. The tumor contained 17-ketosteroids, .42 mg.; total cholesterol, 507 mg.; and free cholesterol, 373 mg./100 Gm.

A 16-year-old woman had amenorrhea, and virilization. Twenty-four-hour urinary

excretion values were: 17-ketosteroids, 12–90 mg.; androgen, 5–35 I.U.; pregnanediol 21–24 mg.; and formaldehydogenic corticosteroid .4–.5 mg. Cortisone therapy caused no improvement. An arrhenoblastoma was surgically removed. It contained: free cholesterol, 3.4 mg. per 100 Gm.; total cholesterol 8.3 mg.; total 17-ketosteroids 9.5 mg.; and 17-ketosteroids after Girard's separation, 3.7 mg./100 Gm. No Δ 3,5 androstadienone-17 was found.

97. A Case of Adrenal Hyperplasia with Unusual Findings.

James T. Bradbury, Sc.D. and *William G. Slate, M.D.* (by invitation).

State University of Iowa, Iowa City, Iowa.

A 22-year-old student appeared because of five years of secondary amenorrhea and hirsutism. Her 17-ketosteroid excretion values were found to be 140 to 190 mg. per twenty-four hours. The Willard Allen test (presumably a test for dehydroisoandrosterone) gave positive results, but intravenous pyelograms and perirenal air studies failed to reveal any adrenal enlargement. Cortisone administered intramuscularly and then orally promptly reduced her 17-ketosteroids and maintained them at normal levels. Menstrual regularity was restored. However, the Willard Allen (blue chromogen) reactions were always positive. Chromatographic separation on an alumina column revealed that at least 2 of the 17-ketosteroid fractions contained blue chromogens. The first fraction off the column contained a compound that was not dehydroisoandrosterone and represented from 20 to 25 per cent of the total ketosteroids in this patient. The ketosteroids in this first fraction were also very low in androgenic potency.

Several peculiarities of this case are presented:

1. The initial amenorrhea was refractory to cyclic stilbestrol therapy in doses as high as 50 mg. per day.
2. The presence of at least two 17-ketosteroids which give positive results with Willard Allen tests in the absence of any demonstrable adrenal tumor.
3. The persistence of regular menstrual periods for six months after cessation of cortisone therapy, in spite of 17-ketosteroid excretion levels ranging from 40 to 60 mg. per day.

98. Failure of Methylandrostenediol to Inhibit Adrenal Suppression by Cortisone in the Human.

Herbert S. Kupperman, Rita Finkler and *Jay Burger* (by invitation).

Diagnostic and Endocrine Laboratories and Newark Beth Israel Hospital, Newark, N. J.; and the Dept. of Therapeutics, New York University-Bellevue Medical Center, New York, N. Y.

Suppression of 17-ketosteroid excretion in the adrenogenital syndrome has been well established. Quantitative relationships between the dose of cortisone administered and the degree of suppression of 17-ketosteroid excretion has been noted. Concomitant administration of methylandrostenediol (MAD) and cortisone to rats resulted in a partial reversal of cortisone-induced adrenal atrophy. Using patients with well recognized adrenogenital syndrome, cortisone and MAD were administered alone and concomitantly. 17-Ketosteroid studies were made during the period of administration. Cortisone, 37.5 mg. administered in divided doses three times a day, effectively suppressed the excessive

ketosteroid excretion in these patients to values well within normal levels. Administration of 25 mg. of MAD twice a day failed to modify the cortisone-induced 17-ketosteroid inhibition. Administration of MAD to a series of aged males with little androgenic function of the testes resulted in a slight decrease of the 17-ketosteroid excretion. The data in the adrenogenital syndrome and in aged males indicate that MAD is ineffective in nullifying cortisone-induced suppression of adrenocortical function as measured by the 17-ketosteroid excretion and does not stimulate adrenocortical function when administered alone.

99. Need for Further Study As To The Role of the Parathyroids in Cushing's Syndrome.

William W. Robinson, M.D. (introduced by George W. Thorn, M.D.)
Rockwood Clinic, Spokane, Wash.

Case Report: A 38-year-old white male was in the terminal stages of Cushing's syndrome, as manifested by severe osteoporosis with multiple spontaneous fractures, "moon-face," purple abdominal striae, hypertension, and extensive arterial calcification. A course of 4000 R units of deep x-ray therapy had been given eight months previously to the pituitary area without benefit (1938). Upon x-ray examination, changes seen in the skeleton and the arterial calcification present were suggestive of hyperparathyroidism. The neck was explored (1939) and three hyperplastic parathyroid glands were removed. There was a drop in the blood calcium level from 11 to 6.25 mg. per 100 ml., and a sudden remission of all the clinical manifestations of classic Cushing's syndrome. The patient, after fifteen years, is well, and symptom-free, with no evidence of recurrence. His skeleton has recalcified and his previous arteriosclerotic arterial system is apparently being slowly decalcified. His blood pressure has dropped from 210/110 to 116/80. His blood calcium level for the past fifteen years has varied from 5.75 to 6.25 mg. per 100 ml. There have been only minimal symptoms which have been easily controlled by a high-calcium, low-phosphorus diet. This case suggests a relationship of parathyroids and the pituitary-adrenal axis. More studies are needed.

100. Studies on the Mechanism of Stimulation of ACTH Secretion: The Blocking Effect of Morphine.

F. Norman Briggs, Ph.D. and *Paul L. Munson, Ph.D.*

Biological Research Laboratories, Harvard School of Dental Medicine, and the Dept. of Pharmacology, Harvard Medical School, Boston, Mass.

The usual adrenal ascorbic-acid response of the intact rat anesthetized with Nembutal to such stimuli as histamine, vasopressin, and laparotomy can be blocked by morphine (Briggs and Munson: *J. Pharmacol & Exper. Therap.* **110**: 7, 1954). However, morphine does not interfere with the normal response to exogenous ACTH, and therefore appears to have the same effect on the release of ACTH as certain lesions in the hypothalamus. Additional experiments on the effect of morphine on the adrenocortical response to epinephrine and to unilateral adrenalectomy are given in the table on the next page.

If we may assume that morphine does not act directly on the adenohypophysis but rather on some part of the central nervous system, then the foregoing results indicate that neither epinephrine nor a subnormal blood concentration of adrenocortical hormones (such as may follow unilateral adrenalectomy) stimulate ACTH secretion by direct action on the pituitary.

Treatment	No. of rats	Morphine* (mg.)	Adrenal ascorbic acid (mg./100 ml.)
A. Epinephrine, 40 μg.*	9	0	325±22
B. Saline	9	0	406± 6
C. Epinephrine, 40 μg.*	8	2	400±11
D. Saline	8	2	424±7
			Significance A *vs.* C $p = <0.01$
E. Unilateral adrenalectomy	13	0	296±22
F. Sham adrenalectomy	15	0	328±12
G. Unilateral adrenalectomy	11	2	375± 8
H. Sham adrenalectomy	14	2	399± 9
			Significance E *vs.* G $p = <0.001$

* All doses are given per 100 Gm. body weight.

101. Apparent Identity of Electrocortin With the Sodium-Retaining Corticoid Extracted From Human Urine.

J. A. Luetscher, Jr., M.D., B. B. Johnson, M.D. (by invitation), *B. J. Axelrad, M.D.* (by invitation), *J. E. Cates, M.D.* (by invitation) and *G. Sala, M.D.* (by invitation).

The Dept. of Medicine, Stanford University School of Medicine, San Francisco, Calif.

A corticoid of high sodium-retaining activity has been found in normal human urine. Active material appears in increased amounts during restriction of dietary sodium. In heart failure, nephrosis, and some related conditions associated with reduced urinary excretion of sodium, greatly increased amounts of the sodium-retaining corticoid appear in the urine. Extraction of the active substance from urine is enhanced by reduced pH or by beta-glucuronidase.

The biologic activity of this corticoid has been compared with that of electrocortin prepared by Tait and Simpson from adrenocortical extract. Both materials are at least twenty times as active as DCA in reducing sodium output and increasing potassium in the urine of adrenalectomized rats. The small doses used affect mineral excretion without significant change in creatinine clearance in the adrenalectomized rat.

The sodium-retaining corticoid resembles electrocortin in ultraviolet absorption at 240 mμ, in infrared absorption characteristic of a Δ^4-3-keto-20-ketosteroid, in tetrazolium reduction, and in chromatographic behavior in various solvent systems. Acetylation of either compound reduces its effect on mineral excretion in rats.

The sodium-retaining corticoid of human urine appears to be identical with electrocortin in these chemical, physical and physiologic properties.

102. High Electrolyte Activity of Some Compounds Related to 11-Desoxycorticosterone.

B. B. Johnson, M.D. (by invitation), *B. J. Axelrad, M.D.* (by invitation), *J. E. Cates, M.D.* (by invitation) and *J. A. Luetscher, Jr., M.D.*

The Dept. of Medicine, Stanford University School of Medicine, San Francisco, Calif.

The potent sodium-retaining hormone from the adrenal cortex ("electrocortin") and from human urine ("sodium-retaining corticoid") has a potency more than twenty times greater than DCA on sodium and potassium excretion in the adrenalectomized rat. In a search for chemical structures which enhance effects on electrolyte excretion, tests have been made on a series of adrenocortical steroids and a number of related substances not previously studied.

Thirty-six substances have been assayed in water-loaded adrenalectomized rats. Observations on the excretion of sodium, potassium and water have been made. The activity of each compound has been compared with that of DCA as a standard.

Assays of new compounds confirm current opinion that reduction of the Δ^4 or 3-keto structure or modification of the α-ketol side chain results in loss of effect on sodium and potassium balance.

The introduction of a hydroxyl group at 2β, 6α, 6β, 11β, 16α or 17α results in reduced electrolyte activity as compared with DCA.

Four compounds have been found to show activity significantly greater than that of DCA. These compounds have in common some modification of the conventional structure about carbons 9, 10 or 11, with loss of hydrogen or of the 19-methyl group, or new substituents.

103. Enhancement of Activity of Hydrocortisone by Substitution of a Halogen Atom in the 9-Alpha Position.

Grant W. Liddle, M.D., Maurice M. Pechet, M.D. (by invitation) and *Frederic C. Bartter, M.D.*

National Heart Institute, Bethesda, Md.

Previous work has indicated that among the synthetic steroids allied to the adrenal the most potent "salt-retaining" steroid was desoxycorticosterone (DOC), and the most potent "glucocorticoid" was hydrocortisone (F).

In the present study, however, it was found that hydrocortisone bearing a chlorine atom in the 9-alpha position (9-alpha-chloro-F acetate, Merck) was more active than DOC in sodium retention, and more active than F in "glucocorticoid" activity.

Bioassays of both properties were performed, using adrenalectomized dogs.

In doses of 25 to 400 micrograms, both DOC and chloro-F acetate induced decreases in sodium and increases in potassium excretion. Renal clearance data indicated altered rates of tubular transport of sodium and potassium. Chloro-F acetate was 3.3 times (1.9–5.2) (95 per cent confidence limits) as active as DOC, but F and F acetate had no consistent sodium-retaining effect.

Chloro-F acetate in doses of 500 to 2,500 micrograms and F acetate in doses of 5,000 to 20,000 micrograms induced eosinopenia. Chloro-F acetate was 8.3 (5.0–13.9) times as active as F. This confirms and extends the work of Fried who found chloro-F acetate to be four times as active in glycogen deposition as cortisone acetate.

Clinical studies have confirmed the potency of 9-alpha chlorohydrocortisone acetate.

104. Simple Nonchromatographic Methods for Specific Estimation of Urinary Metabolites of Hydrocortisone, Corticosterone and 17-OH-Progesterone.

William Q. Wolfson, M.D., with the technical assistance of *Mrs. Mioko Eya-Chambliss, Mrs. Ann L. Grimes-Traverso* and *Miss Wilma Barth.*

The Unit for Metabolic Research, Dept. of Medicine, Wayne University College of Medicine and City of Detroit Receiving Hospital, Detroit, Mich.

The normal level for urinary 17-ketogenic corticoids is 12.5 mg./day (new Zimmermann chromogen as DHA after $NaBiO_3$). This equals butanol-extractable Porter-Silber chromogen, and is about half of butanol-extractable BTZ chromogen. This difference is due to corticoid metabolites, since administered hydrocortisone equalizes BTZ and P-S values. Urinary metabolites of hydrocortisone (17,21-OH-20-ketocorticoids) equal PS *plus* (17-ketogenic minus P-S)when the latter difference does not exceed 50 per cent of an absolutely elevated P-S value. 17-OH-progesterone (17-OH-21-H-20-ketocorticoids) metabolites, except as just noted, equal (17-ketogenic minus P-S). Corticosterone (17-H-21-OH-20-ketocorticoids) metabolites equal (BTZ minus 17-ketogenic) when 17-ketogenic exceeds P-S because of 17-OH-progesterone metabolites; otherwise corticosterone metabolites equal (BTZ minus P-S). A rough assignment of Pettenkofer chromogens and 17-ketosteroids to proper precursors is also possible because hydrocortisone selectively increases the latter, corticosterone increases the former, and 17-OH-progesterone increases neither. Using these techniques, administration of 17-OH-progesterone, hydrocortisone, and corticosterone increase the excretion of only the specific fraction to which their metabolites belong. Normal adult urine contains an average of 25 mg./day of hydrocortisone metabolites, 22 mg./day of corticosterone metabolites, and no significant amount of 17-hydroxyprogesterone metabolites.

105. Metabolites of Hydrocortisone and Cortisone in Synovial Fluid in Rheumatoid Arthritis.

Hildegard Wilson, Ph.D., Richard Fairbanks (by invitation) and *Dominick Scialabba, B.A.* (by invitation).

The Dept. of Chemistry and the Study Group on Rheumatic Diseases, New York University College of Medicine, New York, N. Y.

We have previously reported that rheumatoid synovial tissue metabolizes corticosteroids (*Proc. Soc. Exper. Biol. & Med.* **83**: 648). Doses of 100 mg. of free hydrocortisone (F) and cortisone (E) were injected intra-articularly. Metabolites in synovial fluid were then investigated. Appreciable amounts of F were found after injection of E. The patterns of metabolites of F and E were strikingly different. Since E is not anti-inflammatory in the knee, the local effectiveness of F may therefore reside in the production of a specific metabolite rather than in the unaltered steroid. The chief metabolites, in the order of mobility on toluene-propylene-glycol papergrams were: 1) three hours after E—F, nonreducing material reacting like a 17-ketone, and an α-ketol moving near B (not B); 2) three hours after F—only a 17-hydroxy-α-ketol moving near E (not E); and 3) one-half an hour after F—a Δ^4-3-ketonic 17-hydroxy-α-ketol of extremely low mobility (slower than tetrahydro F), an α-ketol moving near B (not B), Δ^4-androstene-11β-ol-3, 17-dione, and another 17-ketosteroid. Characteristics were deduced from reduction spot tests, Porter-Silber reactivities, absorption spectra in ethanol and in H_2SO_4, and micro 17-KS assays before and after oxidation with HIO_4 or CrO_3.

106. Conjugated Serum Corticoids.

R. Klein, M.D., C. Papadatos, M.D. (by invitation), *J. Fortunato, M.S.* (by invitation) and *C. Byers, B.S.* (by invitation).

Children's Hospital of Pittsburgh and the Dept. of Pediatrics, University of Pittsburgh Medical School, Pittsburgh, Pa.

A technic was devised whereby acid hydrolyzable conjugated serum 17,21-dihydroxy, 20-ketosteroids could be measured. Essentially, it consists in acid hydrolysis of the residue obtained after the free serum corticoids are extracted and measured by the method of Nelson and Samuels. After this residue has been hydrolyzed it is extracted and the corticoid measured as in the original Nelson and Samuels method.

Normal values range from 0 to 25 micrograms per 100 ml. with an average of 8 gamma per 100 ml. The average free corticoid in this series was 13 gamma per 100 ml.

When the level of free corticoid rises in the serum following stress, the administration of ACTH, or the administration of cortisone, the level of bound corticoid also rises. This persists after the elevation of free serum corticoid disappears.

Measurements of both the free and bound corticoid levels were made before cortisone ingestion and at different intervals of time thereafter at points from one to eight hours. Average curves were then drawn. The peak for the concentration of conjugated serum corticoids is lower and occurs later than the peak for free serum corticoids. About three hours after the ingestion of cortisone the two levels are approximately equal. At eight hours the free corticoid falls below the precortisone level and the bound corticoid level is higher than that of either the free corticoid at eight hours or the bound corticoid before cortisone.

107. Plasma 17-Ketosteroids: An Improved Method for Their Extraction and Determination.

George W. Clayton, M.D. and *Alfred M. Bongiovanni, M.D.*

Dept. of Pediatrics, Johns Hopkins University, School of Medicine, Baltimore, Md.

As it has been suggested that steroid hormones are transported in the plasma in close association with protein or lipoprotein complexes, studies have been undertaken to define more clearly this steroid-protein association as it relates to androgenic hormones. During the course of these studies, a method has been found whereby the 17-ketosteroids may be extracted from the plasma without the use of strong hydrolytic procedures. This extraction method employs an organic solvent system and has consistently produced a better yield of steroid than other methods tried. It is also felt that when this method of extraction is used, subsequent hydrolysis of the 17-ketosteroid conjugates is greatly facilitated. By using this extraction procedure and employing a less vigorous method of hydrolysis, the formation of artifacts is prevented and characterization of the 17-ketosteroids of the plasma is facilitated. This method of extraction of the plasma 17-ketosteroids is described. The results obtained when glucuronidase, dioxane or adjustment of the pH to 1 are used to hydrolyze the ketosteroid conjugates are compared with the results employing stronger hydrolytic procedures. Data are also presented pertaining to the manner in which steroid hormones are transported in the circulation.

108. Human Corticosterone Metabolism. IV. Probable Predominance of Corticosterone in the Adrenocortical Secretion of Premature Infants.

J. Frederick Eagle, M.D. (by invitation) and *William Q. Wolfson, M.D.*

The Dept. of Pediatrics, University of Buffalo School of Medicine and the Statler Research Laboratories, Children's Hospital, Buffalo, N. Y.; and The Unit for Metabolic Research, Dept. of Medicine, Wayne University College of Medicine and City of Detroit Receiving Hospital, Detroit, Mich.

The ratio of urinary Pettenkofer chromogens to urinary Zimmermann chromogens probably reflects the ratio of adrenal secretion of corticosterone and hydrocortisone. In female adults this ratio is 0.35, and after ACTH it rises to 0.47. In untreated premature infants the ratio is 2.81, and after ACTH it falls to 0.89, although both moieties increase. Only the administration of corticosterone or dehydrocorticosterone to adults has produced equally high values. This ratio is not due to increased excretion of dehydroepiandrosterone. Paper chromatograms show no significant DHA in these urines, but much material consistent with corticosterone metabolites.

The data suggest: 1) the basal corticosterone/hydrocortisone ratio in the premature infant is higher than that in adults; 2) ACTH increases both corticosterone and hydrocortisone secretion, but the B/F ratio is higher in prematures than in adults; 3) there is no physiologic adrenal insufficiency or unresponsiveness in premature infants; 4) persistence of the premature type of adrenal secretion into early childhood can explain the characteristic low 17-ketosteroid output which is not accompanied by adrenal insufficiency; and 5) it may be unnecessary to postulate an adrenarche.

109. Plasma 17-Hydroxycorticosteroid and Blood Cell Changes in the Rhesus Monkey After Whole Body X-Irradiation.

Arthur B. French, M.D. (by invitation), *Claude J. Migeon, M.D.* (by invitation), *Leo T. Samuels, Ph.D.* and *John Z. Bowers, M.D.*

The Radiobiology Laboratory and the Depts. of Biological Chemistry and Medicine, University of Utah College of Medicine, Salt Lake City, Utah.

Plasma 17-hydroxycorticosteroid levels and hematologic changes were followed in 24 rhesus monkeys after acute whole body x-irradiation. The steroid response varied with the radiation dose from a minimal change after 50 r, to a maximal rise after doses of 400 r or more. After 400 to 800 r there was a rapid rise in steroid levels to a peak averaging 84 μg./100 ml. after six hours. The normal average at this time was 26 μg./100 ml. By twelve hours after irradiation the steroid levels had fallen to normal. If death impended there was a progressive rise during the last few days to a terminal average of 291 μg./100 ml. This preterminal increase reached levels higher than those found after maximal ACTH stimulation and suggests the presence of a regulatory factor in addition to ACTH.

With 800 r, eosinophil counts fell abruptly from a control average of 407 per cu. mm. to a minimum of 8 per cu. mm. at eight hours after irradiation. The eosinophil counts then rose to normal at twenty-four hours and gradually decreased as pancytopenia appeared. Total leucocyte and polymorphonuclear counts showed an early rise paralleling the steroid rise.

The effect of shielding and other operative procedures on these changes is discussed.

110. Excretion of Hydrocortisone-4-C^{14}.

Paul M. Hyde, Ph.D. (by invitation) and *Robert H. Williams, M.D.*

University of Washington School of Medicine, Seattle, Wash.

Previous studies have demonstrated variations in the metabolism of steroids, depending upon the route of administration. That circulation through the liver exerts an important influence on the fate of hydrocortisone is indicated by significant A-V differences of hepatic blood and by a retention of the hormone in the blood after hepatectomy. In order to extend these studies, a dose of 50 micrograms of hydrocortisone-4-C^{14} (4.5×10^5 d/m) was given intragastrically, intramuscularly or intravenously to normal Sprague-Dawley male rats. More than half of the radioactivity given by any of these routes was found in the feces of normal animals during the first four days of the experimental period; the remainder was in the urine. Bile from rats in which the bile ducts were cannulated contained approximately 60 per cent or more of the administered C^{14} after radiohydrocortisone was given by the three routes. In all experiments with animals having bile fistulas, absorption and excretion into the bile occurred rapidly with about 20 per cent or more of the C^{14} given being excreted during the first four hours. The absence of significant amounts of fecal radioactivity after intragastric intubation of radioactive compound F indicated almost complete absorption from the gastro-intestinal tract. Higher concentrations of radiocarbon were found in the urines of normal animals than in the urines of animals with cannulated bile ducts, suggesting that enterohepatic circulation takes place with hydrocortisone-4-C^{14} or its radiometabolites.

111. The Histochemical Reaction of Connective Tissue After the Local Administration of Adrenal Steroids.

Rex Bigler, M.D. (by invitation), *Hilton A. Salhanick, Ph.D.* and *Thomas F. Dougherty, Ph.D.*

The Dept. of Anatomy and the Dept. of Obstetrics and Gynecology, University of Utah College of Medicine, Salt Lake City, Utah.

The histochemical reaction of Seligman and Ashbel for ketosteroids (*Endocrinology* **50:** 338, 1952) was used to study the distribution of hormones in loose connective tissue. Subcutaneous tissue spreads from control mice injected locally with physiologic saline solution consistently showed a few small, positive-staining granules in eosinophils and fibroblasts, and dense granulation of the mast cells. Other cells gave no reaction. The local injection of 10–25 μg. of hydrocortisone resulted in a heavy collection of purple granules, indicating a positive ketosteroid reaction in the fibroblasts but not in other constituents of the connective tissue. The reaction was present two hours after injection and remained for five to ten days. Cortisone, Reichstein's substance S (17α-hydroxy-11-desoxycorticosterone), 11-desoxycorticosterone and, to a lesser degree, progesterone and corticosterone, as well as the acetates of these compounds, gave positive results. The glucuronide of 11-desoxycorticosterone, however, gave negative results. Kendall's compound A acetate (11-dehydrocorticosterone-21-acetate), tetrahydrocortisone-21-acetate, tetrahydrocortisone, and tetrahydrohydrocortisone did not elicit the formation of granules. Ethanol extraction prior to reaction with 2-hydroxy-3-naphthoic acid hydrazide indicated a decrease in positive staining substance. It cannot yet be concluded that the granules reflect intracellular localization of steroid hormones and not reactive metabolic ketones resulting from the action of these hormones, but experiments in progress designed to elucidate this phase and utilizing C^{14}-ring-labeled hormones will be discussed.

112. Histologic Study of Testes at Puberty. II. Histochemistry.

R. E. Mancini, M.D. (by invitation), *F. A. de la Balze, M.D.* (by invitation), *Clorinda J. Scarpa, M.D.* (by invitation), *R. Lobo, M.D.* (by invitation) and *J. Andrade, M.D.* (by invitation).

III Catedra de Clinica Medica de la Facultad de Medicina, Buenos Aires, Argentina.

A histochemical study* of the testis was made in 11 cases. Results follow:

1. *Tubule wall.* In the basal membrane, reticular fibers were condensed and glycoproteins and mucopolysaccharides appeared; fibrocyte nuclei accumulated alkaline phosphatase. In the tunica propria, mucopolysaccharides and elastic fibers appeared, and reticular fibers increased and became organized. In mature testes some fibroblasts had alkaline phosphatase, and the cytoplasm contained ascorbic acid, lipids and ribonucleoproteins.

2. *Intertubular connective tissue.* Simultaneously with testicular maturation, lipids and ascorbic acid appeared in the cytoplasm of the differentiated fibroblasts, and alkaline phosphatase in their nuclei. Some of the intermediate cells exhibited a small quantity of lipids, ribonucleoproteins and ascorbic acid in the perinuclear zone of the cytoplasm, and alkaline phosphatase in the nucleoli. The cytoplasm of the mature and immature Leydig cells accumulated large quantities of ascorbic acid, lipids and ribonucleoproteins. Numerous mature Leydig cells accumulated cholesterol esters. Involutive cells showed ascorbic acid, lipids and ribonucleoproteins in the cytoplasm. In the rest of the intertubular connective tissue reticular fibers were irregularly distributed or tended to surround Leydig cells. Amorphous substance reacted positively to the riboglycoprotein method and diminished as maturity developed.

All changes described were progressive. Findings in the intratubular content are presented.

This study has enabled us to suggest the probable origin and evolution of Leydig cells.

* 1. PAS reaction, controlled by ptyalin, hyaluronidase and methanol chloroform digestion. 2. Metachromatic technique with Azure B, controlled by hyaluronidase digestion and perchloric acid pretreatment. 3. Ascorbic acid reaction of Deane and Morse. 4. Sudan Black B, Nile Blue sulphate and Schultze reaction. 5. Kabath and Furth method for alkaline phosphatase. 6. Del Rio Ortega's silver impregnation and Weigert's elastic stain.

113. Factors Influencing Adrenocortical Function as Studied in Guinea Pigs.

Robert S. Ely and *Vincent C. Kelley.*

Salt Lake County General Hospital, Salt Lake City, Utah.

Since certain problems concerned with adrenocortical function cannot be answered satisfactorily in human subjects, a suitable experimental animal is highly desirable. The guinea pig, a common small laboratory animal in which adrenal steroid production is similar to that in man, seems most suitable. In guinea pigs adrenal ascorbic-acid concentration and circulating concentrations of 17-hydroxycorticosteroids were measured following hypophysectomy. From initial levels, several times those found in man, the 17-hydroxycorticosteroid values decreased rapidly during the first few hours and thereafter stabilized. However, following adrenalectomy they decreased to zero within twenty-four hours. Salicylate administration in intact guinea pigs resulted in elevated plasma steroid concentrations. This response did not occur in hypophysectomized or adrenalectomized

animals. Guinea pigs on a scorbutogenic diet had normal steroid levels; these increased tenfold with the development of clinical scurvy, and then decreased in a few hours following a single injection of ascorbic acid. Before the steroid levels became elevated the adrenals were nearly devoid of ascorbic acid, suggesting a poor correlation between the two.

The responses of adrenal ascorbic acid and plasma steroid concentrations to exogenous ACTH were studied in normal, scorbutic, and hypophysectomized guinea pigs. These results are discussed with regard to the possibility of applying the ACTH-steroid response in hypophysectomized guinea pigs as an ACTH bioassay technic.

114. Role of Various Steroids in Effecting Survival After Several Types of Stress in the Adrenalectomized Mouse.

Francis J. Saunders, Ph.D.

Division of Biological Research, G. D. Searle & Co., Chicago, Illinois.

Adrenalectomized mice were subjected to various types of stress. The protective actions of cortisone acetate and desoxycorticosterone were studied in all cases. In some experiments the effects of estrone, testosterone propionate, epinephrine and certain synthetic compounds were also followed. Stress, which involves hyperkaliemia, was induced either by injection of KCl or by administering large doses of glucose by stomach tube. In both cases, cortisone acetate and desoxycorticosterone were equally effective. In stress involving hypoglycemia (insulin administration) or exposure to cold, cortisone acetate was effective, whereas desoxycorticosterone was inactive. Cortisone acetate also afforded protection against histamine shock, anaphylactic shock, egg-white toxicity and snake-venom poisoning, whereas desoxycorticosterone did not. The effects of other drugs are discussed.

115. Metabolic Relation of Ascorbic Acid, Glutathione and Adrenocortical Hormone in Survival of Mice During Cold Stress.

Walter M. Booker, James R. Tureman (by invitation), *Frances DaCosta* (by invitation), *John Poulson* (by invitation) and *Samuel Mitchell* (by invitation).

Dept. of Pharmacology, Howard University Medical School, Washington, D. C.

Ascorbic acid and glutathione are both effective in increasing the survival time of intact mice subjected to cold stress, but are poorly effective in adrenalectomized mice, in which condition it is well known that adrenocortical hormones are effective. Varying doses of glutathione (2 mg. to 200 mg. per 100 grams of body weight) were tried on intact and adrenalectomized mice. Ascorbic acid was used in a dosage of 20 to 100 mg. per 100 grams of body weight. Cortisone was used in a dosage of 1 mg. per 100 grams body weight. It appears that combinations of ascorbic acid and cortisone are more effective in increasing the survival time of adrenalectomized mice than combinations of glutathione and cortisone. Likewise the oxygen uptake is greater in the cortisone or cortisone-ascorbic acid groups than in the glutathione and glutathione-cortisone groups. Liver tissue and whole body oxygen uptake experiments show that ascorbic acid, glutathione and cortisone are more effective in increasing the oxygen uptake in intact animals than in adrenalectomized animals at room temperature. It is of great interest that adrenalectomized mice subjected to cold show great increases of oxygen uptake over their unstressed controls, but their short survival in cold suggests a "limited metabolic reserve."

Experiments show that glutathione acts to keep ascorbic acid in its reduced state; but it appears that in the absence of the adrenal hormone, ascorbic acid is very ineffective from a metabolic point of view in increasing the survival of animals subjected to cold.

116. Undernourished Patients Treated with Cortisone; Remarkable Effects in Anorexia Nervosa.

Manuel M. Villaverde, M.D.

Hospital Ntra. Sra. de las Mercedes, Habana, Cuba.

Cortisone proved useful in the treatment of undernourished patients. Ten patients who did not react to other treatment received cortisone orally, in divided doses ranging from 19.25 mg. to 100 mg. daily. The diagnoses were: 1) visceroptosis and hyperestrogenism; 2) Sjogren's syndrome and hypo-ovarianism; 3) idiopathic hypoplasia; 4)Basedowoid neurosis; 5) mild hyperthyroidism (2 cases); 6) anorexia; 7) hyperestrogenism and pre-diabetes; and 8) anorexia nervosa (2 cases).

There were no undesirable side effects, even when continued for twelve to thirty-six weeks. Blood potassium concentration was normal in all instances when tested.

In practically all cases, both appetite and weight increased, and an additional remarkable psychologic effect was noted in the 2 patients with anorexia nervosa. Best results were obtained when a dose of 75 to 100 mg. was given daily. Hyperthyroid patients react better to 75-mg. doses than to 100-mg. doses.

117. Effects of Carbonic Anhydrase Inhibitor in Addison's Disease.

George J. Hamwi, M.D. and *David B. Brown, M.D.* (by invitation).

Division of Endocrinology and Metabolism, Dept. of Medicine, The Ohio State University Health Center, Columbus, Ohio.

Carbonic anhydrase inhibitor (CAI), acetazoleamide, was administered to 2 addisonian patients in order to determine the possible interrelationships in the kidney between the carbonic anhydrase mechanism and the adrenocortical hormones in their regulation of the urinary electrolyte excretion. Each patient was maintained on a constant carbohydrate, fat, protein and salt intake. Adrenal replacement therapy was withheld for at least one week prior to starting the study. Twenty-four-hour urines were collected and the urinary volume, pH, specific gravity, sodium, potassium, and chloride were determined. Serum values for sodium, potassium, chloride, and CO_2 combining power were also obtained. The effects of CAI were recorded before and after adrenal hormone replacement therapy was instituted. The adrenal hormones administered were hydrocortisone in one period and a combination of DCA and cortisone in two periods.

The administration of CAI resulted in an increase in the urinary volume and the total excretion of sodium, potassium and chloride, and an elevation of the pH before and after treatment with the adrenal steroids. Minor quantitative but no qualitative differences were noted between the effects of compound F and a combination of DCA and cortisone after the administration of CAI. These results point to the fact that the carbonic anhydrase mechanism can operate in the absence of the adrenal hormones. Furthermore, the ion exchange mechanism controlled by the adrenal steroids can operate in the presence of CAI.

Since CAI produced the signs and symptoms of adrenal insufficiency in both patients prior to steroid replacement therapy, the possibility of using CAI as a diagnostic test for Addison's disease is suggested. It is also postulated that a defect in the carbonic an-

hydrase mechanism may be a cause of the "salt-losing syndrome," since it was shown that the inhibition of carbonic anhydrase produces a diuresis of sodium and chloride independently of adrenal steroid activity.

118. Effects of Cortisone, DCA, ACTH, and Adrenalectomy on Gastric Pepsinogen and on Intestinal Motility.

D. H. P. Streeten, M.R.C.P., B. I. Hirschowitz, M.R.C.P. and *H. M. Pollard, M.D.* (introduced by Jerome W. Conn, M.D.).

The Gastrointestinal Research Division, Dept. of Internal Medicine, University of Michigan Medical School, Ann Arbor, Mich.

When compared with no treatment in sham-adrenalectomized control rats:

1. Cortisone (25 mg. daily) strikingly reduced appetite, body weight, gastric pepsinogen and intestinal propulsive motility;
2. Desoxycorticosterone acetate (5 mg. daily) decreased body weight but produced statistically significant increases in both gastric pepsinogen and bowel motility;
3. ACTH gel (10 units daily) increased pepsinogen but only slightly, and inconsistently accelerated motility; and
4. Bilateral adrenalectomy had no significant effects.

Groups 1 and 2 were adrenalectomized, and group 3, sham-adrenalectomized; all were injected for one week. Rats in all groups except group 1 were individually pair-fed with group 4. Severe anorexia in the rats given cortisone prevented pair-feeding in group 1, but normal, untreated rats pair-fed with this group showed none of the effects produced by cortisone. The index of intestinal motility used was the percentage of small bowel traversed by dyes administered before killing the animals. The pepsinogen content of the homogenized whole stomach was determined by the hemoglobin method.

Similar studies on guinea-pigs revealed a pronounced stimulant effect of ACTH on bowel motility but no significant effect of cortisone or DCA. The action of hyper- and hypo-adrenocortical activity on gastric secretion and small bowel transport in man is discussed.

119. Sexual Development in Cortisone-Treated Female Rats.

Ben B. Blivaiss, Ph.D., Russell O. Hanson, Ph.D. (by invitation), *Robert E. Rosenzweig, B.S.* (by invitation) and *Katherine McNiel, B.S.* (by invitation).

Dept. of Physiology and Pharmacology, The Chicago Medical School, Chicago 12, Illinois.

Sexual development was studied in 24 pairs of pair-fed female rats given daily 1 mg. of cortisone acetate subcutaneously starting at 23 days of age. This dose retarded gain in body weight and produced a significant reduction in weights of adrenals and thymus. There were no marked changes in fasting blood glucose, NPN, or urinary nitrogen. There was a marked increase in ovarian and uterine weights ($p = <0.001$) after two or four weeks of treatment, compared to controls. This increase tended to regress in animals sacrificed three weeks after cessation of treatment. Increased weight of ovaries appears to be due to greater follicular development. No marked histologic differences were evident in the uterus. Opening of the vagina was delayed for four to twelve days, animals treated for four weeks showing longer delay. The gonadotropic hormone content of pituitaries from rats treated for two or four weeks appeared greater than in controls when assayed by the increase in ovarian weights ($p = <0.001$). Uterine weights were increased in re-

cipients of pituitaries from two-week treated animals, but decreased when donors were treated for four weeks. When the same experiment was performed with animals fed *ad libitum*, results were similar to the foregoing but had a lesser degree of difference.

120. Effect of Cortisone on the Distribution of Radioiodine in Control, Thyroxine and TSH-Treated Rats.

D. M. Woodbury, Ph.D., D. T. Goates, B.S. (by invitation), *G. R. Lee, B.S.* (by invitation), *C. B. Williams, B.S.* (by invitation), *P. S. Callister, B.S.* (by invitation) and *J. E. Craighead, B.S.* (by invitation).

Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah.

Previous investigations in this laboratory have shown that, in TSH-treated hypophysectomized rats, cortisone decreases the 24-hour thyroidal uptake of I^{131} and the plasma I^{131} concentration, and increases urinary excretion of I^{131}. In order to elucidate further the effects of cortisone on thyroid function the following experiments were performed. I. The effects of cortisone on BMR and on I^{131} distribution in control, thyroidectomized and thyroxine-treated rats were measured. Cortisone increased the BMR of control and thyroidectomized rats; the increased BMR produced by thyroxine was decreased by cortisone. Urinary I^{131} excretion was increased by cortisone in control, thyroidectomized and thyroxine-treated animals. II. The effects of cortisone on I^{131} distribution were measured in untreated and TSH-treated hypophysectomized rats and in untreated and TSH-treated hypophysectomized, bilaterally nephrectomized rats. Cortisone markedly *decreased* thyroidal I^{131} uptake in TSH-treated hypophysectomized rats but markedly *increased* uptake in both untreated and TSH-treated hypophysectomized-nephrectomized animals. Cortisone decreased plasma I^{131} in all groups. In addition, the gastrointestinal tract content of I^{131} in untreated and TSH-treated hypophysectomized-nephrectomized rats was increased by cortisone. It is thus evident that cortisone stimulates the thyroid gland, but that this effect is masked by its action in increasing I^{131} in the urine and enteric contents. Nephrectomy unmasks the stimulating effect of cortisone on the thyroid.

121. Non-Parallel Changes in the Cholesterol and Ascorbic Acid Content of the Adrenals of Malaria-Parasitized Chicks.

D. J. Taylor, M.S. (by invitation), *J. Greenberg, Ph.D.* (by invitation), *E. S. Josephson, Ph.D.* (by invitation) and *E. M. Nadel, M.D.*

National Institutes of Health, Bethesda, Maryland.

The stress of malarial infection in chicks is associated with adrenal enlargement in the absence of a decrease in the ascorbic-acid content of the gland (Nadel *et al.;* Josephson *et al.; J. National Malaria Soc.* **8:** 70, 132, 1949). Others have since reported the observation of this difference in the response of avian adrenals to stress. Studies correlating adrenal histochemical sudanophilia and chemical analysis of cholesterol, were undertaken in control and in experimentally infected (Pl. gallinaceum) chicks. For histochemical studies, adrenal glands from experimental and control birds were paired, and were sectioned, stained and mounted on the same slide together. For chemical analysis, adrenal glands were dried over sulfuric acid *in vacuo* to constant weight prior to analysis in 4 series including 47 birds. The adrenals of parasitized chicks were larger than in their nonparasitized controls and, when examined chemically, contained normal amounts of

ascorbic acid. The parasitized chicks had adrenals with significantly less total cholesterol (0.47 ± 0.07 *vs.* 0.74 ± 0.03 Gm./100 Gm. of tissue), less cholesterol ester (0.45 ± 0.07 *vs.* 0.73 ± 0.02 Gm./100 Gm. of tissue), and less sudanophilic material, as determined histochemically. These data support the view that in this species the metabolism of adrenal ascorbic acid and cholesterol may not be directly interrelated.

122. Effect of Hyper- and Hypoadrenocortical Function on Young Fat-Deficient Male Rats.

John C. Finerty, Ph. D. and *Theodore C. Panos, M.D.*

University of Texas Medical Branch, Galveston, Texas.

Male Holtzman weanling rats were grouped as follows: 41 were given a synthetic fat-free diet and 32 were fed Purina Laboratory Chow. Of the former, 20 were adrenalectomized and maintained on 1 per cent saline, and 11 received cortisone subcutaneously, 2 mg./100 Gm. daily. Of the chow-fed group, 12 were adrenalectomized and 10 received cortisone. After sixteen days, the endocrine glands, liver, heart, kidneys and blood were taken for study. The fat-free diet alone caused retarded growth and increase in relative weight of brain, kidneys, liver and heart. The fat-free animals tolerated adrenalectomy as well as those fed chow, and in both groups, the relative weight of the brain was increased equally. Expected relative enlargement of liver, heart and kidneys in response to fat deficiency did not occur in the fat-free adrenalectomized group. Cortisone caused pronounced growth retardation, especially in the fat-free group, and relative enlargement of the brain, liver, kidneys and heart. Enlargement was most pronounced in the liver and brain of the fat-free group. Relative enlargement of the testes in response to cortisone occurred in the chow group, but not in the fat-free rats.

123. Adrenal Pituitary Response to 30 Per Cent CO_2 in Air and in Oxygen.

C. T. G. King, Ph.D. and *K. E. Schaefer, M.D.* (introduced by Joseph Aub, M.D.)

U. S. Naval Medical Research Laboratory, New London, Conn.

Normal, adrenalectomized, and hypophysectomized male rats of the Wistar-Hisaw strain (70 ± 10 days of age) were exposed to 30 per cent CO_2 in air and in oxygen for a period of one hour. Autopsy was performed one hour after exposure. White blood counts, differential, absolute eosinophil, absolute lymphocyte counts and total blood sugar determinations were carried out before, during and after exposure. Additional data were obtained from the adrenal cholesterol of guinea pigs submitted to the same experimental procedures.

As a result of exposure to 30 per cent CO_2 in air, hypercapnia and anoxia developed, but 30 per cent CO_2 in O_2 produced only hypercapnia. Both experimental conditions produced in rats a significant leucocytosis (+50 per cent) and lymphocytosis, which were eliminated in hypophysectomized but not in adrenalectomized rats. The number of eosinophils in the normal rat under CO_2 in O_2 rose to 206–209 per cent; this effect was eliminated by adrenalectomy or hypophysectomy. An increased adrenocortical activity was indicated by a marked eosinopenia and lymphopenia in rats and guinea pigs one hour after exposure to CO_2 in air and in O_2, which was eliminated in the adrenalectomized rat under CO_2 in O_2. Additional evidence was found in the decreased adrenal cholesterol content in guinea pigs.

124. Carbohydrate Metabolism and Adrenocortical Activity Under 30 Per Cent CO_2 in Air and Oxygen.

K. E. Schaefer, M.D. and *C. T. G. King, Ph.D.* (introduced by Gregory Pincus).

U. S. Naval Medical Research Laboratory, New London, Conn.

Inbred guinea pigs of the Connaught strain were exposed to 30 per cent CO_2 in air and in oxygen over periods of ten and sixty minutes. Measurements were taken of CO_2, O_2 content of whole blood, hematocrit, pH, serum potassium, blood sugar and lactic acid content. Total blood counts, liver and muscle glycogen and adrenal cholesterol were determined. The two experimental conditions produced the same degree of acidity of the blood at the end of one hour's exposure (pH = 6.86 and 6.87), the difference being an additional anoxia under CO_2 in air. Under 30 per cent CO_2 in air the adrenal cholesterol content decreased at ten and sixty minutes of exposure. Leukocytes, absolute number of eosinophils and lymphocytes did not change significantly. Under CO_2 without anoxia (70 per cent O_2) adrenal cholesterol did not change, nor did eosinophils and lymphocytes. CO_2, both with and without anoxia produced decreased liver glycogen (−52 per cent −62 per cent) and an increase in blood sugar (+96 per cent +95 per cent). With CO_2 and anoxia, muscle glycogen increased 32 per cent and lactic acid decreased 50 per cent; under CO_2 without anoxia, opposite changes were found (muscle glycogen −35%, and lactic acid +42 per cent).

125. The Permissive Role of Adrenal Steroids in Protein Anabolism.

Norman D. Lee, Ph.D.

Veterans Administration Medical Teaching Group Hospital, Memphis, Tenn.

The intraperitoneal administration of *l*-tryptophan to the adult albino rat results in a marked increase in liver tryptophan peroxidase activity within one hour. Enzyme activity values become maximal within two to three hours and return to baseline levels within twelve hours. Furthermore, this phenomenon is accompanied by changes in liver protein turnover, as determined with S^{35}-labeled cystine, and may be blocked by the previous administration of ethionine, a structural analog of methionine. Consequently, these findings make reasonable the assumption that the increase in liver tryptophan peroxidase activity represents specific protein synthesis. In the adrenalectomized, salt-maintained rat this rapid increase in enzyme can still be elicited, although to a reduced degree. In addition, substances (epinephrine and histamine) producing nonspecific enzyme synthesis in the intact rat are no longer effective. The subcutaneous administration of minimal doses of aqueous adrenocortical extract (0.25 ml.) to the adrenalectomized rat restores the capacity for tryptophan peroxidase synthesis, whereas larger doses (3.0 ml.) are ineffective. These results are in agreement with similar experiments involving net nitrogen metabolism, and support the thesis that adrenocortical steroids exert a permissive role in protein anabolism.

126. A Histologic Study of the Effects of Cortisone on the Lower Incisors of Newborn Rats.

E. D. Goldsmith, Ph.D. and *Leonard Ross, M.S.* (by invitation).

College of Dentistry, New York University, New York, and Medical College and School of Dentistry, University of Alabama, Birmingham, Alabama.

As a result of macroscopic observations Parmer *et al.* (1951) reported a cortisone-in-

duced acceleration of tooth eruption in the newborn rat. The present report concerns itself with a histologic study of the effects of cortisone on the dental structures. Daily subcutaneous injection of cortone (Merck), 0.1 mg./day divided into two equal doses, was begun in 24-hour-old rats; littermates served as aqueous vehicle-injected controls (0.02 cc./day). Animals were sacrificed at 3, 5, 7, 9 and 11 days of age. Seventy experimental and 40 control animals were used, and 35 representative lower jaws were prepared for histologic examination. A marked developmental precocity could be seen as early as the third day of age in the experimental group. At this time, the advancing tip of osteodentin is larger than in the controls and has already entered the basal layer of the oral epithelium. By the fifth day, the tip of the lower incisor in the experimental animals is almost completely through the oral epithelium which is completely keratinized in this region. By the seventh day, the tip of the tooth is well into the oral cavity. This condition does not obtain in the controls until three days later. At all ages studied, the cortisone-treated animals exhibited a progressive reduction of the periodontal connective tissue elements. Odontogenesis and amelogenesis were accelerated in the cortisone-treated rats; the odontoblasts and ameloblasts were, however, typical in appearance.

127. Potentiating Effect of Cortisone (E) on the Diuretic Action of Choline Theophyllinate (CT).

Thomas H. McGavack, M.D., Helmut Haar, M.D. and *Sidney Pearson, Ph.D.*

The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

Two men, aged 45 (Subject A) and 65 (Subject B) respectively, without active, acute or chronic illness, were selected at random for this study from the patients on the wards of the Research Unit. Intake of food, fluid and sodium chloride was standardized. After a "fore" period of from one to several weeks, they were subjected (fasting) on test days to a water load of 1000 cc. to be drunk in a half hour. Urinary volume, sodium, chloride and potassium excretion were measured for 24-hour periods. On test days, separate determinations were carried out for four periods as follows: 9 A.M. to 1 P.M., 1 P.M. to 5 P.M., 5 P.M. to 9 P.M. and 9 P.M. to 9 A.M. On the first test day, water alone was used. On successive test days the following were given, respectively: 1) water alone; 2) water plus CT (1200 mg. in divided doses); 3) water plus E (in divided doses of 50, 100 and 200 mg.); and 4) water plus CT plus E. Water alone caused a diuresis of water, sodium, and potassium in both subjects. CT alone increased excretion of water, sodium, potassium and chloride in A, but in B had no effect upon water and actually caused retention of sodium, potassium and chloride. When CT and E were used in combination, there was a marked increase in both A and B in the excretion of water, sodium and chloride with all levels of dosage. This change was most marked for sodium. Potassium excretion was directly proportional to the dose of E used. E, when used in conjunction with CT following a water load, exerted a marked additive effect upon the diuresis of water, sodium, potassium and chloride.

128. Influence of Desoxycorticosterone Acetate (DCA) and Cortisone (E) Upon Water and Electrolyte Excretion in Man.

Helmut Haar, M.D., Sidney Pearson, Ph.D. and *Thomas H. McGavack, M.D.*

The New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York 17, N. Y.

Two men, aged 45 (Subject A) and 65 (Subject B) respectively, without active, acute or chronic illness, were selected at random for this study from the patients on the wards of the Research Unit. Intake of food, fluid and sodium chloride was standardized. After a "fore" period of from one to several weeks, they were subjected (fasting) on test days to a water load of 1000 cc. to be drunk in a half hour. Urinary volume, and sodium, chloride and potassium excretion were measured for 24-hour periods. On test days, separate determinations were carried out for four periods as follows: 9 A.M. to 1 P.M., 1 P.M. to 5 P.M., 5 P.M. to 9 P.M. and 9 P.M. to 9 A.M. On the first test day, water alone was used. On successive test days the following were given, respectively: 1) water alone; 2)DCA—10 mg. at 10 P.M. the day before, and at 6 A.M. the day of the test; 3) E—50, 100 and 200 mg. in equally divided doses, at 10 P.M. the day before, and at 6 A.M. the day of the test; and 4) DCA plus E, in the varying doses mentioned. Water alone caused a diuresis of water, sodium, chloride and potassium in both subjects. DCA decreased the excretion of water, sodium and chloride and increased the excretion of potassium. In relation to E administration, the 2 subjects behaved somewhat differently. In both subjects, E in doses of 50 and 100 mg. respectively showed no appreciable influence on the excretion of water. When the dosage was raised to 200 mg., water excretion was furthered in A and not appreciably altered in B. In A, the excretion of sodium, potassium and chloride was increased at all dosage levels. In B there was no change in sodium and chloride excretion in the two smaller doses, with a marked decrease when a dose of 200 mg. was employed. Potassium excretion was decreased in B at all three levels of dosage. E in 25-mg. doses almost completely neutralized the water-retaining effect of DCA, and partially its sodium and chloride-retaining effect. In dosages of 50 mg., the 2 subjects behaved differently. In A, the DCA-retaining effect on water, sodium and chloride was partially neutralized, whereas in B there was an additive retention of all three materials. The use of 50 mg. of E in conjunction with DCA, furthered potassium excretion in A, whereas in B no additive action was noted. From these studies, it is concluded that DCA and E may, in the same dosage, act differently at different ages. It is further concluded that E in moderate dosages acts antagonistically to DCA except in the excretion of potassium. In larger amounts, it shows an additive influence upon water, sodium and chloride retention.

PITUITARY

129. The Renal Action of the Parathyroids.

Frederic C. Bartter, M.D.

The Clinic of General Medicine and Experimental Therapeutics, National Heart Institute, Bethesda, Md.

There is at present no consensus as to the mode of action of parathyroid hormone on the kidneys. Parathyroid extract has been reported to produce 1) no primary renal effect, inorganic phosphate (P) diuresis, if any, being secondary to a rising level of serum P, 2) P diuresis resulting from nonspecific increases in glomerular filtration rate (GFR), and 3) P diuresis resulting from decreases in tubular reabsorption.

The problem was reinvestigated in unanesthetized fasting parathyroidectomized dogs. Elevated serum creatinine levels were maintained by constant intravenous infusion. After control periods a "primer" of parathyroid extract (Lilly) was given intravenously, and subsequent infusion solution adjusted to deliver 5–10 units a minute. Arterial blood was drawn at midpoints of urine periods, which were terminated by triple washings. Filtered P was estimated as GFR $\times$ (serum P).

Parathyroid extract produced 1) slight to moderate increases in GFR, 2) prompt ten-to twenty fold increases in urine P excretion, and 3) prompt decreases in concentration of serum P. By adjusting dosages it was possible to arrange that total filtered P showed no appreciable rise throughout the studies. Thus all the observed changes could be accounted for by decreases of tubular reabsorption. Urinary P loss, at first closely commensurate with estimated total loss from extracellular fluid, later exceeded this figure as P entered extracellular fluid from tissues.

130. Use of Ethylenediamine Tetra-acetate in the Measurement of Parathyroid Gland Function.

G. S. Stewart, Ph.D.

Rush Department of Biochemistry, The Presbyterian Hospital of the City of Chicago, in affiliation with the University of Illinois College of Medicine, Chicago 12, Illinois.

Ethylenediamine tetra-acetate (EDTA) was injected intravenously at a dosage level of 40 mg./Kg. into 5 dogs several days before operation, immediately following thyro-parathyroidectomy, and five days following the operation. Urinary calcium was measured for the five-hour observation period and changes in extracellular fluid (ECF) calcium were estimated from the initial and final serum calcium levels. Calcium mobilized, presumably from bone, was determined by difference. The results, in terms of mg. of calcium per Kg. of body weight, are tabulated:

	Before op.	Immediately following op.	Five days after op.
Urinary Ca	3.86	3.48	3.70
Change in ECF Ca	−0.13	−2.06	−1.64
Ca from bone	3.73	1.42	2.06

The ability of EDTA to induce a consistent loss of calcium from the body, and the large reduction in both ECF and mobilized calcium in the animals which were operated on, indicate the spectral range of parathyroid gland dysfunction that one might anticipate would be capable of differential analysis, especially at subclinical levels. Clinical evaluation of this parathyroid gland function test is at present in progress and is discussed.

131. Changes Observed After Hypophysectomy in 4 Patients Suffering from Advanced Cancer of the Breast.

Jose A. Schutte, M.D. (by invitation), *Jorge A. Picaza, M.D.* (by invitation), *Zoilo Marinello, M.D.* (by invitation) and *Juan F. Marquez, M.D.* (by invitation).

Instituto del Radium, Hospital Mercedes, Habana, Cuba.

Four patients suffering from advanced mammary cancer have been hypophysectomized during the year 1953. All of them were given androgens and x-ray therapy some time before their operations. Stilbestrol was used in 2 cases. At the time this abstract is being written, Patient No. 2 is dead, but Patients 1, 3, and 4 are still living twelve, nine and five months, respectively, after their operations. Three of them were operated upon when

they were in a terminal status and no therapy was effective in limiting the progression of the metastases. After hypophysectomy, Patient No. 1 showed such a regression of the metastases that about 95 per cent of them disappeared. Patient No. 2 remained unchanged for a month, then became worse and died two weeks later. Patient No. 3 showed a remarkable reduction in metastases of the lung, but cerebral metastases became worse. These facts were recognized from the fourth month after surgery up to now. Patient No. 4 is under close observation to determine the exact situation, though she gives the impression of 30 per cent improvement. All these facts may be verified on the slides or film that accompany this report.

132. Serum Inorganic Phosphate Response in Pituitary Disorders.

J. Schiller, M.D. and *E. A. Tyan, M.A.* (by invitation).

Hospital Pitié, Paris, France.

There is a definite correlation between the glucose tolerance curve and that of the serum inorganic phosphate in the normal individual. High glycemic levels are correlated with low inorganic phosphate levels, the reverse being true for low glycemic levels. This is best demonstrated in gastrectomized patients with hypoglycemic disorders in which extreme values are obtainable. This pattern of response is not usual in patients with pituitary disorders. Since an elevated level of serum inorganic phosphate is not always found in acromegaly, the possibility that a curve would furnish more conclusive data was considered. Concomitant glucose tolerance (A-V) and inorganic phosphate curves were obtained in acromegalic patients and the data showed: 1) high inorganic phosphate levels (over 3.8 mg.) at all glycemic levels over a three-hour period, and 2) an increased instead of a decreased phosphatemia, regardless of the presence or absence of carbohydrate intolerance. These findings were in contrast to those obtained in patients with hypopituitary disorders, in whom the shape of the curve was the reverse of that found in acromegaly, *i.e.*, the inorganic phosphate level remained low (below 3 mg. per 100 ml. and in 1 case below 2 mg. per 100 ml.) at all glycemic levels. Similar results were obtained in patients with anorexia nervosa, which could be considered a hypopituitary condition secondary to nutritional deficiency.

133. Effect of Hypophysectomy on Neoplastic Disease in Man.

Olof H. Pearson, M.D., Bronson S. Ray, M.D. (by invitation), *Charles G. Harrold, M.D.* (by invitation), *Charles D. West, M.D., Min C. Li, M.D.* (by invitation) and *John P. Maclean, M.D.* (by invitation).

Memorial Center and New York Hospital, New York, N. Y.

Hypophysectomy has been carried out in 18 patients with advanced neoplastic disease during the past two years. Three patients have shown objective evidence of tumor regression after hypophysectomy.

A woman with metastatic mammary carcinoma, who had previously undergone castration, adrenalectomy and androgen therapy, obtained further objective improvement from hypophysectomy. This improvement has lasted six months. Administration of somatropin for eighteen days apparently accelerated the growth of osseous metastases which subsided when the somatotropin was withdrawn. The data suggest that pituitary somatotropin is an important factor for mammary carcinoma.

A man with metastatic prostatic cancer, previously orchiectomized, obtained objective improvement after hypophysectomy. The improvement has lasted four months,

and is manifest by a fall in acid phosphatase to normal levels, regression of the prostatic mass and improvement of paraplegia due to tumor invasion of the spinal canal.

A woman with metastatic malignant melanoma showed marked shrinkage of subcutaneous tumor masses during a four-week period after hypophysectomy. This patient apparently suffered severe anoxia during hypotensive anesthesia, which resulted in acute renal damage and severe cerebral damage. That anoxia may have played a role in the tumor regression in this case cannot be excluded.

Eight patients died within three weeks of surgery. Three patients failed to show improvement but hypophysectomy was incomplete. In 4 patients the effect cannot yet be evaluated.

134. Effect of Hypophysectomy Upon Cyclic Mitotic Activity in the Retromolar Mucosa of Rats.

Helmut A. Zander, D.D.S., Jens Waerhaug, D.D.S. and *Franz Halberg, M.D.* (all by invitation).

The Division of Periodontology, School of Dentistry, University of Minnesota, Minneapolis, Minn.

Fifty sham-hypophysectomized rats and 50 hypophysectomized rats were maintained, singly housed, under controlled environmental circumstances. Four months after the operations one half of each group was killed during the day and the other half during the night. Completeness of hypophysectomy was authenticated in sections of sella turcica. The numbers of mitoses/2000 epithelial cells/rat were determined in sections of retromolar mucosa (which was consistently free of inflammation). Mitotic rates in night samples from both groups were similar (2.74 ± 0.44 mitoses per 2000 cells examined, for the hypophysectomized rats; and 2.72 ± 0.41 mitoses, for the shami-hypophysectomized rats; $p = 0.98$). The corresponding day values were 6.13 ± 0.64 for the hypophysectomized rats, and 10.95 ± 1.03 for the sham-hypophysectomized rats. Statistical analyses of the data substantiate: 1) the findings of a mitotic cycle in hypophysectomized as well as in sham-hypophysectomized rats (p's < 0.0001), 2) the amplitude of the mitotic cycle is reduced at four months after hypophysectomy ($p = 0.0002$) and 3) the reduction in amplitude of the cycle is brought about by a lower mitotic rate in hypophysectomized rats ($p < 0.0001$) at the daily peak time of mitotic activity in sham-hypophysectomized rats.

135. Hyperphagia, Obesity and Duodenal Ulcer Associated with Hypothalamic Leukemic Infiltration.

Robert P. Heaney, M.D. (by invitation), *Leonard P. Eliel, M.D., Walter Joel, M.D.* (by invitation) and *Hugh Stout, M.D.* (by invitation).

The Oklahoma Medical Research Foundation and the University of Oklahoma School of Medicine, Oklahoma City, Okla.

Marked hyperphagia, occurring suddenly during an aminopterin-induced remission in a 5-year-old female child with acute lymphatic leukemia, resulted in a weight gain of 4 kilograms over a two-week period (an increase of 20 per cent above her previously well-nourished level). The resulting obesity was confined chiefly to face, trunk, and upper arms. There was generalized hirsutism, but no hypertension, glycosuria or polyuria; 17-ketosteroid and 11-oxysteroid excretions remained normal. Her abnormal weight level was maintained until death, six weeks after the onset of over-eating, and twenty-

two months after the onset of leukemia. During this period, despite continued evidence of apparent hematologic remission, she manifested repeated marked elevations of cerebrospinal fluid pressure and progressive hepatosplenomegaly. Death was due to a medullary pressure cone syndrome. Autopsy revealed in addition to generalized leukemic infiltration, a subacute, penetrating, duodenal ulcer and marked leukemic infiltration of the hypothalamus. The sudden, dramatic change in this child's eating habits and weight curve corresponds exactly to the behavior of rats subjected to electrolytic destruction of the ventromedian nuclei of the hypothalamus. It may be anticipated that similar complications will be encountered with increasing frequency as chemotherapy increases the longevity of patients with acute leukemias.

136. Correction of Malignant Exophthalmos by Transection of the Pituitary Stalk After Unsuccessful Orbital Decompression.

R. W. Schneider, M.D. and *W. James Gardner, M.D.* (by invitation).

Cleveland Clinic, Cleveland, Ohio.

In June 1953, a 65-year-old diabetic woman first noted onset of exophthalmos with periorbital edema and blurring of vision. A subtotal thyroidectomy had been performed twenty years earlier, and she had remained euthyroid in the intervening years. Localized myxedema appeared on the anterior surface of both legs four years earlier. Rapid progession of the exophthalmos occurred within the next four months, with bilateral choked discs, and marked impairment in vision. A bilateral orbital decompression was successfully performed in October, with only transient improvement in vision, followed by continuing advancement in all of the signs of malignant exophthalmos to the point of almost complete blindness. Two and a half months later, a second craniotomy was performed; the pituitary stalk was observed to be markedly hypertrophied and about equal in size to the internal carotid artery. The stalk was severed from the pituitary and rapid improvement in the exophthalmos followed within the course of the next two weeks. The accompanying colored photographs show the remarkable improvement in the exophthalmos, before and after the second operation.

137. Administration of Intravenous Calcium to Aid in the Diagnosis of Hyperparathyroidism.

R. W. Schneider, M.D.

Cleveland Clinic, Cleveland, Ohio.

At the annual meeting of this Society in June 1952, Howard and co-workers described their results with the use of intravenous calcium as a measure of activity in the parathyroid glands. Evidence at that time suggested its value in the diagnosis of hyperparathyroidism. In the intervening period, the author has had the opportunity to employ this test, following the described procedure (*J. Clin. Endocrinol. & Metab.* **12**: 927, 1952), in 20 patients with results in close agreement to those reported.

For purpose of interpretation, the patients were divided into two groups: those with a 24-hour urinary calcium excretion over 150 mg., and those with an excretion under 150 mg. The group with hypercalciuria responded to intravenous calcium with a rise in the level of urinary and serum phosphorus or a fall in serum phosphorus. In all instances, either adenoma or hyperplasia was found at operation. In only 1 patient of this group did an exception occur, namely, a fall in urinary phosphorus (associated with a fall in the level of serum phosphorus). After two adenomas were removed from the para-

thyroids of this patient, hyperparathyroidism persisted, and repetition of the test gave similar results, following which a third adenoma was removed.

Those patients with normal calcium excretion showed less consistent results—usually a fall in urinary phosphorus and a rise in level of serum phosphorus. Only 1 of the patients without hypercalciuria has been explored; no tumor was found.

Final conclusions regarding the value of this test for recognition of hyperparathyroidism cannot be made at this time, but the results in general agree with those of earlier investigators.

138. Production of Polycythemia in Hypophysectomized Rats by the Pituitary Erythropoietic Factor.

A. N. Contopoulos, M.D. (by invitation), *S. Ellis, Ph.D.* (by invitation), *M. E. Simpson, M.D.* (by invitation), *J. H. Lawrence, M.D.* (by invitation) and *H. M. Evans, M.D.*

Institute of Experimental Biology and The Donner Laboratory, University of California, Berkeley, Calif.

A severe and progressive anemia resulting in a 50 per cent decrease in hematologic values develops within sixty days after hypophysectomy. Repair of this anemia has been reported after injection of sheep anterior pituitary extracts. Stimulation of circulating erythrocytes above the level found in normal animals (polycythemia) has not hitherto been reported in hypophysectomized rats following the administration of any known agent, including the pituitary hormones, its target organ hormones, or cobalt. However, preparations have now been separated from glacial acetic-acid extracts which, when given to hypophysectomized rats two months postoperatively for fourteen days in 100-μg. daily doses increased the hematocrit and total red cell volume (Fe^{59} method) 110 per cent above the values in the hypophysectomized controls, and 45 per cent above normal values.

139. Hormonally-Induced Lactation in Male Rats.

Wm. R. Lyons, M.D., Choh Hao Li, Ph.D., Ruth E. Johnson, B.A. (by invitation) and *Roger D. Cole, B.S.* (by invitation).

The Dept. of Anatomy, School of Medicine, the Dept. of Biochemistry and the Institute of Experimental Biology, University of California, Berkeley, Calif.

At the 1953 meetings we presented methods for inducing mammary growth and lactation in virgin rats with pituitaries, gonads and adrenals removed. It is now possible to report the same results in immature male rats following a similar procedure. The 26-day-old Long-Evans male rat has little more than a rudimentary mammary apparatus, and it was necessary to prolong the first or developmental phase of treatment to two or three weeks in order to imitate the gland of pregnancy. The procedure was: 1) hypophysectomize; 2) three days later remove adrenals and testes; 3) immediately after second operation begin daily subcutaneous injections of 10 I.U. of estrone, 4 I.U. of progesterone, 60 I.U. of mammotropin, and 0.25 mg. of somatotropin; 4) inject intraperitoneally daily 3.0 cc. of 5 per cent glucose in 0.85 per cent NaCl; 5) use 1 per cent NaCl in the drinking water.

The rats fared well on this regimen; their weight increased 2–3 Gm. per day, and their mammary glands showed good lobulo-alveolar development.

During the second or lactational phase (five days or less) 1 mg. of hydrocortisone was injected daily, estrone and progesterone were withheld, and the doses of mammotropin and somatotropin were doubled. At necropsy the males' nippleless glands were found engorged with milk.

140. Localization of Radioactivity in the Rat Prostate After the Administration of Labeled Prolactin Preparations.

Martin Sonenberg, M.D., Ph.D., William L. Money, Ph.D. and *Rulon W. Rawson M.D.*

Sloan-Kettering Institute of the Memorial Center for Cancer and Allied Diseases, New York, N. Y.

An anterior pituitary preparation containing prolactin has been labeled with radioactive iodine. The biologic activity of prolactin in this labeled preparation has been retained. When this material was injected into male rats, the distribution of radioactivity was similar in comparable tissues to that noted in female rats (*Endocrinology* **49**: 709, 1951). There was a large concentration of radioactivity in the prostate following injection of the labeled preparation, with the maximum localization at 100 minutes after intracardiac administration. The concentration was greater than in blood and many other tissues. No other radio-iodine-labeled substances, hormonal or nonhormonal, showed the same degree of localization of radioactivity in the prostate. Castration, ten days or more prior to the administration of labeled prolactin preparations, resulted in a greater prostatic localization of radioactivity, with a maximum concentration being realized 300 minutes rather than 100 minutes after injection. The disappearance of radioactivity from blood was slower in the case of the castrated rats. Pretreatment of animals with estradiol enhanced the prostatic concentration of radioactivity. Similar treatment of rats with testosterone appeared to depress the localization of radioactivity in the prostate after administering radioiodinated prolactin preparations.

141. Further Observations Regarding the Effects of Pituitary Growth Hormone in Human Subjects.

Gilbert C. Cochrane, M.D., George Fukayama, B.A., George D. Michaels, Ph.D., Harry E. Balch, M.D. and *Laurance W. Kinsell, M.D.*

Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, Calif.

A variety of growth hormone preparations have been evaluated in this laboratory since the first report of an anabolic effect produced by one specific preparation. These subsequent studies indicate that the problem of consistent production of pituitary material which is significantly anabolic in human subjects and totally free of untoward effects, is still unsolved in a chemical sense.

Most preparations which have been used, when administered either intravenously or intramuscularly have some degree of pyrogenic effect, which for obvious reasons makes quantitative evaluation of anabolic activity impossible. One observation has so far been consistent—that anabolic effects in humans have been obtained only with materials which are soluble at a physiologic pH. Elevation of the respiratory quotient, and a fall in iodine number of the plasma occur during pituitary growth hormone administration. The possible implications of these findings are of interest.

142. *In Vivo* Effects of Growth Hormone on Transaminases and *l*-Glutamic Acid Dehydrogenase.

Oliver H. Gaebler, M.D. and *Alex Zuchlewski, B.S.* (by invitation).

Edsel B. Ford Institute for Medical Research, Detroit, Mich.

Glutamic alanine transaminase in liver, aspartic glutamic transaminase in liver and muscle, and *l*-glutamic acid dehydrogenase in liver, were determined in hypophysectomized rats. The control series received injections of saline and the treated series, 100 gamma of growth hormone per rat per day. Animals were sacrificed after 0, 2, 5, and 10 days of treatment, to secure data throughout the period when nitrogen storage is induced. Previous studies of these enzymes in this laboratory and elsewhere involved large doses of growth hormone or a single period of observation. Significant findings in growth hormone-treated rats may be summarized as follows: 1) Changes in *l*-glutamic acid dehydrogenase were not remarkable; 2) Glutamic alanine transaminase activity in liver fell throughout the 10-day period, to a level only 25 per cent of that in saline controls; 3) Glutamic aspartic transaminase activity in liver decreased at first, but returned to the control level in 10 days, whereas in muscle it fell slowly and remained significantly low at the end of the 10-day period. Experiments with still smaller doses of growth hormone will be undertaken to determine whether the striking changes in transaminase activity always occur during induced growth.

143. A Technique for Quantitative Evaluation of the Effects of Anabolic and Catabolic Hormonal Agents.

George D. Michaels, Ph.D., Etsuko Osawa, B.S., Charles Baker, M.D. and *Laurance W. Kinsell, M.D.*

Institute for Metabolic Research of the Highland Alameda County Hospital, and the Dept. of Pathology, Samuel Merritt Hospital, Oakland, Calif.

Following the implantation of sterile gauze pledgets under the abdominal skin of adult rats, fibroplasia occurs at a predictable rate, in terms of histologic and chemical evaluation. In control animals, the pledgets, when removed at three-day intervals over a thirty-day period, have a predictable weight within a relatively narrow range and a predictable content of nitrogen, phosphorus, potassium, sodium, sulfur and isotopic sulfur. (A tracer dose of S-35 labeled methionine is administered twenty-four hours before autopsy.)

Impregnation of the pledgets with specific amounts of such hormonal agents as hydrocortisone acetate, pituitary growth hormone, insulin, testosterone, and other anabolic steroids, results in significant changes in the weight and in the qualitative and quantitative composition of the pledgets.

By impregnating pledgets on one side and using untreated pledgets on the other, one can evaluate in a relative and absolute fashion both local and systemic effects of the hormone used for impregnation. Data available to date indicate profound inhibition of fibroplasia with hydrocortisone, in association with marked increase in sodium and decrease in potassium content of the newly formed tissue; a significant local and systemic anabolic effect with pituitary growth hormone; and a possible stimulating effect upon fibroplasia with very small doses of hydrocortisone. Data regarding other hormonal agents are still too few to permit of critical evaluation, but will be reported in full later.

144. Effect of Hypophysectomy and Growth Hormone on Serum Polysaccharide Level in the Rat.

M. R. Shetlar, Ph.D. (by invitation), *Clara L. Shetlar, B.S.* (by invitation) and *R. W. Payne, M.D.*

The University of Oklahoma School of Medicine, Dept. of Biochemistry and Pharmacology, and the Research Laboratory, VA Hospital, Oklahoma City, Okla.

Serum polysaccharide-protein and serum mucoprotein-polysaccharide concentrations were determined in intact animals by previously described methods (*Cancer Res.* **10**: 681, 1950).

After hypophysectomy a decrease in the level of serum polysaccharide occurred. When growth hormone was administered to these animals there was an elevation of serum polysaccharide, frequently to levels higher than those of the corresponding control animals. Testosterone propionate, desoxycorticosterone acetate and cortisone acetate in dose of 1 mg. daily for twenty-eight days and corticotropin given in a dose of 0.5 mg. daily for twenty-eight days produced no change in the serum polysaccharide level of hypophysectomized rats. Growth hormone administration to intact rats was followed by a slight increase in serum polysaccharides. When subjected to stress, by the intramuscular injection of 0.5 cc. of turpentine, hypophysectomized rats showed a rise in the level of serum polysaccharides comparable to that in intact rats. In confirmation of a previous report (*Am. J. Cancer* **32**: 50, 1938), the Walker 256 tumor grew at a greatly reduced rate in hypophysectomized rats; an elevation of the serum polysaccharide level occurred in these animals.

The concentration of serum mucoprotein was not affected by hypophysectomy or by growth hormone. Elevated serum mucoprotein levels occurred in hypophysectomized rats bearing Walker 256 tumors and in those injected with turpentine.

145. Effect of Adrenocorticotropic and Growth Hormones on the Muscle Glycogen of Hypophysectomized Rats.

Peter Fonss-Bech and *Choh Hao Li*

Hormone Research Laboratory, Dept. of Biochemistry, University of California, Berkeley, Calif.

Hypophysectomized male rats weighing 250-300 Gm. were fasted for twenty-four hours on the fourteenth postoperative day; during the period of fasting, ACTH in a beeswax-peanut oil suspension was administered subcutaneously in two divided doses. The animals were sacrificed twenty-four hours after the first injection; the gastrocnemius muscles were removed and the glycogen content was estimated by the procedure of Nelson (*J. Biol. Chem.* **153**: 375, 1944). It was found that ACTH is highly active in maintaining the muscle glycogen in fasting hypophysectomized rats. A total dose of 1.0 I.U. of ACTH per 100 Gm. of body weight maintained the glycogen level at 478 mg. per cent, whereas that of the controls decreased to 296 mg. per cent.

With respect to growth hormone (somatotropin) the findings of Russell and Wilhelmi (*Endocrinology* **47**: 26, 1950) on its glycostatic effect have been confirmed. It was shown further that limited hydrolysis of growth hormone with chymotrypsin, which was found earlier not to affect its growth-promoting activity (Condliffe and Li: *Fed. Proc.* **11**: 198, 1952), does not alter its myoglycostatic potency. On the other hand, peptic

digestion of growth hormone abolishes its growth-promoting activity, with an accompanying loss of glycostatic action. There is no evidence to indicate that the growth-promoting and glycostatic activities can be dissociated from the somatotropin molecule.

146. Influence of Growth Hormone (GH) on the Excretion of Water and Electrolytes.

Adrienne A. Batts, M.D. (introduced by Leslie L. Bennett, M.D.)

Dept. of Physiology and the Institute of Experimental Biology, University of California, Berkeley, Calif.

Rats fasted twelve hours were placed in metabolism cages, and given by stomach tube an amount of 0.2 per cent NaCl equivalent to 2.5 per cent of body weight, followed two hours later by twice this volume. Half of the rats also received 500 gamma of GH with the initial hydration. Urine volume was measured every fifteen minutes for four hours, beginning with the second hydration, and the total Na excretion determined. The water excreted by GH-treated rats averaged 86 per cent (range 79.5–91.7 per cent) of that excreted by controls, and the Na excretion averaged 56 per cent (range 51.3–61.8 per cent) of that of the controls. If the animals were hydrated with 0.9 per cent NaCl the excretion of both water and Na by the GH-treated rats was around 50 per cent of the normal control excretion. The effect of GH was prolonged, inasmuch as the excretion of water and Na by animals injected seventeen hours before hydration was identical with that observed when the injection was made with the initial hydration. Additional experiments showed that administration of 1 mg. of GH daily to hypophysectomized rats for at least two days elevates the thiocyanate space (from 31.5 to 35.2 per cent body weight, $p < .01$), and the plasma volume (from 2.82 to 3.15 per cent body weight, $p < .01$), as measured by Fe^{59}-tagged red cells.

147. Effect of Growth Hormone on the Renal Function and Electrolyte Balance in Intact and Hypophysectomized Rats.

Monroe S. Glitzer, Ph.D. and *W. R. Boss, Ph.D.* (introduced by J. H. Birnie).

Dept. of Zoology, Syracuse University, Syracuse, N. Y.

Intact, 7-day and 30-day hypophysectomized rats were treated for five days with either saline, or 0.5, 2.0, and 4.0 mg./Kg./day of purified growth hormone (Armour #R491024). On the terminal day the effects of the hormone were measured by simultaneous renal clearances of creatinine and *p*-aminohippurate, diuretic rates and electrolyte balance. Although the depressed GFR and RPF of all 7-day and 30-day hypophysectomized animals were raised, none of the renal functions was significantly increased in the intact rats. The hormone had a greater reparative effect on restroation of the RPF than on the GFR. In neither the intact nor the hypophysectomized rats did the hormone enhance the excretion of the two hydrating doses of water administered prior to the renal tests. The adrenal/body-weight ratios did not demonstrate the presence of a possible ACTH contaminant. It was found that the hormone at all dosage levels increased the functional capacity of the kidney. The excretion of sodium and potassium by the intact, 7-day and 30-day hypophysectomized rats was not altered by the hormone treatments, with the exception of the 4.0 mg./Kg. dose, which caused a marked excretion of sodium in the 30-day series.

148. Studies of Fluid Metabolism of the Hypophysectomized Rat.

Erich H. Loewy, B.A. (by invitation) and *Charles W. Lloyd, M.D.*

State University of New York, College of Medicine, Syracuse, N. Y.

Hypophysectomized male Sprague-Dawley rats weighing approximately 150 Gm. and intact controls were pair-fed a high calorie, high protein diet which maintained a good growth rate in both groups of animals. Although the hypophysectomized animals were unable to excrete a water load normally, they had a greater spontaneous water intake and output than the controls. Administration of pitressin tannate daily for seven days or more produced a greater decrease in fluid intake and a smaller urine volume in the hypophysectomized than in the normal animals. The decrease in fluid intake occurred more rapidly in the hypophysectomized animals than in the normal rats, so that the latter were in a more positive water balance than the hypophysectomized animals, even though a greater decrease in urine volume was produced in the hypophysectomized animals. The normal group showed a greater decrease in the level of serum Na than did the hypophysectomized animals. The liver of hypophysectomized animals maintained in good nutrition had normal ability to inactivate pitressin *in vitro*, and administration of pitressin tannate for seven days or more before sacrifice did not change this ability. Nutritional deficiency which occurred if the hypophysectomized animals were allowed to eat without restriction produced marked impairment of *in vitro* pitressin inactivation.

149. Rate of Decay of the Antidiuretic Effect of Injected Vasopressin in Rats.

R. H. Kellogg, M.D., Ph.D., H. N. Jacobson, M.D. and *R. R. Newlon, B.A* (introduced by L. L. Bennett, M.D., Ph.D.).

The Dept. of Physiology of the Harvard Medical School, Boston, Mass., and the Dept. of Physiology of the University of California School of Medicine, Berkeley, Calif.

Doses of Pitressin ranging from 0.01 to 1000 milliunits were injected intravenously into unanesthetized male rats during diuresis produced by continuous intravenous infusion of saline at about 0.12 ml./min. The urine flow and chloride concentration were measured at each urination.

Characteristically, Pitressin decreased the urine flow and increased the urinary chloride concentration reciprocally. Only the largest doses increased the rate of excretion of chloride per minute. The length of the period of antidiuresis, or elevated urinary chloride concentration, following each injection was found to be linearly related to the logarithm of the dose of Pitressin injected, except at the extreme ends of the dosage range used. On the assumption that each increment in the duration of antidiuresis represents the time necessary for the corresponding increment in dose to be destroyed *in vivo*, these results indicate that the rate of destruction of antidiuretic activity in the rat is proportional to the amount present, *i.e.*, it decays exponentially, with a half-life of about five minutes. This conclusion has been confirmed by additional experiments in which Pitressin was infused continuously at a steady rate until a balance between intake and destruction had been achieved.

150. Effect of Ascorbic Acid on Insulin-Glucose Tolerance and Lactic-Pyruvic Acid Ratio in Obesity.

E. C. Arendt, M.D. (by invitation) and *C. J. Pattee, M.D.*

Queen Mary Veterans Hospital, Montreal, Canada.

Intermediary metabolism, notably that of the carbohydrates, was studied in the obese patient. Glucose curves obtained in the routine manner were within normal

limits in all but 3 cases. Insulin-glucose tolerance-curves (as described by Engel) in these patients fell into a characteristic pattern, simulating that of curves found in patients with Cushing's syndrome or during cortisone therapy. Blood pyruvate and lactate levels were also determined. The findings are discussed in relationship to hepatic function. Of greater interest is the concept that obese patients, as a result of constant stress, become subclinically depleted of ascorbic acid. This theory is supported by the work of King and coworkers, and Yavorsky. The former showed that, in the guinea pig, impairment of glucose tolerance parallels depletion of ascorbic acid. Obese patients were given large doses of ascorbic acid by oral or intramuscular route. There was a lowering of the intravenous insulin-glucose curve in a significant number of patients. The probable mechanism of action of the vitamin is discussed. Variations of the pyruvic-lactic acid ratio and the normal, before and after ascorbic acid administration, are presented.

151. Comparison of Diabetogenic Action of Cortisone and of Growth Hormone in Different Species.

Karl E. Paschkis, M.D. and *William A. Abelove, M.D.* (by invitation).

Division of Endocrine and Cancer Research, Jefferson Medical College, Philadelphia, Pa.

In cats, cortisone caused mild hyperglycemia. When growth hormone, in subeffective doses, was given simultaneously with cortisone, marked hyperglycemia and glycosuria was observed. In one dog, cortisone caused moderate hyperglycemia and glycosuria; in a second, no response was obtained even with administration of glucose (as drinking solution). In guinea pigs, cortisone cause severe hyperglycemia and glycosuria, but no "metasteroid" diabetes was produced. Growth hormone caused transient hyperglycemia; the diabetogenic effect of growth hormone was not intensified by simultaneous intraperitoneal glucose injections. Combined treatment with cortisone and growth hormone gave no evidence of synergistic action, as seen in the cat. In rabbits diabetes developed under cortisone treatment, but in order to maintain the diabetes, the dose of cortisone had to be gradually increased. Growth hormone caused hyperglycemia but, with the dose employed, no glycosuria. Combined treatment with cortisone and growth hormone yielded no unequivocal evidence of synergism. It would appear that the differences in response to the two diabetogenic hormones in the difference species studied is one of degree. The possible significance of the results with regard to the mode of action of the two hormones is discussed.

152. Comparison of the Effects of Variations in Amino-Acid and Carbohydrate Intake on the Metabolic Response to Cortisone Acetate.

Leonard P. Eliel, M.D. and *Robert P. Heaney, M.D.* (by invitation).

Oklahoma Medical Research Foundation, Oklahoma City, Okla.

The metabolic response to oral cortisone acetate (200–600 mg./day) was studied in a patient fed synthetic diets of differing amino acid and carbohydrate contents. On an amino acid-free diet, nitrogen excretion increased 0.1 Gm./day, whereas a 100-Gm. amino acid isocaloric intake was associated with a nitrogen loss of 1.5 Gm./day, when cortisone was administered. During a period of intense protein anabolism, with a 50-Gm. amino acid intake, negative nitrogen balance was achieved only at high dosage levels of cortisone. Significant rises in urinary calcium, and simultaneous falls in alkaline phosphatase concentrations occurred at all amino acid and carbohydrate levels during

hormone administration. Moderate reduction or doubling of the carbohydrate intake failed to alter significantly nitrogen or calcium balances, upon administration of cortisone. Cortisone-induced eosinopenia and retention of Na, Cl, and water were manifested similarly at all amino acid and carbohydrate levels. The results confirm the antianabolic effect of cortisone on protein metabolism but suggest that small amounts of protein were lost through increased catabolism. The level of amino acid intake, and the presence or absence of protein deficit, rather than the level of carbohydrate intake, appear to determine the effect of cortisone acetate on protein metabolism.

GROWTH HORMONE SYMPOSIUM

A 3-day International Symposium on the *Hypophyseal Growth Hormone, Its Nature and Actions* is being sponsored by the Henry Ford Hospital and the Edsel B. Ford Institute for Medical Research. The Symposium will be held in the New Clinic Building Auditorium of the Henry Ford Hospital, Detroit, Michigan, October 27, 28 and 29, 1954.

Formal presentations and discussions will cover the following aspects of growth hormone investigation: bioassay, preparation and physico-chemical properties of growth hormone; effects of growth hormone on certain structures such as the mammary gland, skeletal system, connective tissue, islets of Langerhans, kidneys, gastrointestinal tract and tissue cultures; growth hormone and energy sources; the effect of growth hormone on cellular systems; the influence of growth hormone on lactation; and its metabolic effects in man.

Interested persons should write the Program Committee, Henry Ford Hospital, Detroit 2, Michigan. Invitations will be sent to as many persons as possible.